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Minireview

Novel inhibitors of advanced glycation endproducts

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Abstract

A number of natural or synthetic compounds as AGE inhibitors have been proposed, discovered or currently being advanced by others and us. We have identified two new classes of aromatic compounds; aryl- (and heterocyclic) ureido and aryl (and heterocyclic) carboxamido phenoxyisobutyric acids, and benzoic acid derivatives and related compounds, as potential inhibitors of glycation and AGE formation. Some of these novel compounds also showed "AGE-breaking" activities in vitro. Current evidence is that chelation of transition metals and/or trapping or indirect inhibition of formation of reactive carbonyl compounds are involved in the mechanisms of action of these novel AGE inhibitors and breakers. Here, we review the inhibitors of glycation and AGE-breakers published to date and present the results of our in vitro and in vivo investigations on a number of these novel AGE inhibitors. These AGE-inhibitors and AGE-breakers may find therapeutic use in the treatment of diseases that AGE formation and accumulation may be responsible for their pathogenesis such as diabetes, Alzheimer's, rheumatoid arthritis, and atherosclerosis.

Keywords: AGE; Glycation inhibitors; Chelating agents; Reactive carbonyl scavengers; Oxidative stress

Deterioration of tissues and organs in several pathological conditions, such as diabetes [1,2], chronic inflammatory diseases [3], Alzheimer's disease [4], atherosclerosis [5,6], and in normal aging [7] has been widely associated with the formation and accumulation of damage from chemical processes induced by oxidation stress, glycation, carbonyl stress, and UV-irradiation [8–10]. All these processes are potent inducers of reactive oxygen species (ROS)[1] and reactive carbonyl species (RCS) [3].

Oxidative stress

> . . .It would appear that life originated as a result of free radical reactions (FRRs), selected FRRs to play major metabolic roles, and used them to provide for aging, mutation, and death, thereby assuring evolution. Further, life span evolved in parallel with the ability of organisms to cope with damaging free radical reactions. In short, the origin and evolution of life may be due to free radical reactions and, in particular, to their ability to induce random change. If so, it is remarkable that life with its beautiful order owes its origin to, and is sustained by, a class of chemical reactions whose outstanding characteristic is their unruly nature. The aging process may be simply the sum of the deleterious FRRs going on continuously throughout the cells and tissues. The process may never have changed; in the

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[1] *Abbreviations used:* 2, 3, DAP, diaminophenzine; 3-DG, 3-deoxyglucosone; AD, Alzheimer's disease; AG, aminoguanidine; AGE, advanced glycation endproducts; ALE, advanced lipoxidation endproducts; ALS, amyotrophic lateral sclerosis; CEL, carboxyethyllysine; CML, carboxymethyllysine; CMV, carboxymethylvaline; DTPA, diethylenetriaminepentaacetic acid; ELISA, enzyme-linked immunosorbent assay; FFI, furoyl-furanyl-imidazole; FL, fructose-lysine; FN3K, fructosamine-3-kinase; GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; GLA, glycolaldehyde; GO, glyoxal; GOLD, glyoxal-lysine-dimer; HFBA, heptafluorobutyric acid; HNE, 4-hydroxy-2-nonenal; HPLC, high performance liquid chromatography; IL, interleukin; MDA, malondialdehyde; MGO, methylglyoxal; MOLD, methylglyoxal-lysine-dimer; NF-κB, nuclear factor-κB; NOS, nitric oxide synthase; PDGF, platelet-derived growth factor; PKC, protein kinase C; PM, pyridoxamine; RAGE, receptor of AGE; RCS, reactive carbonyl species; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFα, tumor necrosis factor α; UCP, uncoupling protein; VEGF, vascular endothelial growth factor.

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beginning the reactions were apparently largely initiated by UV radiation from the sun, and to a lesser extent by volcanic activity; and now they arise from enzymatic and non-enzymatic free radical reactions. Denham Harman [11]

Oxidative stress (Fig. 1) is defined as a situation of serious imbalance between the production of free radicals (ROS), and antioxidant defense mechanisms, leading to potential tissue dysfunction and damage [12,13]. The excess free radical production under hyperglycemic conditions originates from mitochondrial respiration [14], cytochrome p450 [15], xanthine oxidase [16], PKC-dependent activation of NADH/NADPH oxidase [17], and RAGE-triggered cellular oxidant stress [18].

Ever increasing evidence indicates that ROS are specific signaling molecules in both physiological and pathophysiological conditions. The generation of ROS, within certain boundaries, is essential to maintain homeostasis. For example, phagocytic cells generate ROS as an essential mechanism to combat infection [19], or cytosolic ROS produced in response to growth factor stimulation are involved in proliferative response [20]. Regardless of how and where ROS are generated (exogenously or intracellular), a rise in the oxidant level has two important effects: damage to various cell components and triggering the activation of specific signaling pathways (NF-κB, ERK, JNK, NAPK, and PKC isoforms) [21]. Reactive oxygen species encompass a variety of diverse chemical species including superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), alkoxyl ($RO^{\cdot}$), peroxyl ($ROO^{\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and hypochlorous acid (HOCl) [22] (Fig. 1).

Most estimates suggest that the majority of intracellular ROS production is derived from the mitochondria. The production of mitochondrial superoxide radicals occurs at two electron transport chains, namely at complex I (NADH dehydrogenase) and at Complex III (ubiquinone-cytochrome *c* reductase) [23] (for details of the sources of ROS generation, please refer to [19 and 22]. Estimates indicate that, in vivo, mitochondria convert 0.1–1% of the oxygen molecules consumed into superoxide anions [19]. Whatever the absolute amount of mitochondrial ROS, given their potentially harmful effects, numerous protective mechanisms have evolved to limit their production and release. One such mechanism is to increase the rate of metabolic uncoupling (UCP 1,2,3) [24]. When oxygen consumption is uncoupled from ATP generation, heat is produced. The consumption of oxygen without ATP production would also reduce the levels of superoxide generation [19]. The mitochondrial ROS production is largely counteracted by an antioxidant defense system that includes SOD, catalase and glutathione peroxidase enzymes. The balance between ROS production and antioxidant defenses determines the degree of oxidative stress [19,25].

Most recently, new sources of ROS generation have been reported [26–30]. These reactive oxygen species are generated in the early and the advanced glycation processes and these species have been shown to exhibit cytotoxicity. For example, AGE deposited in the arterial wall could generate free radicals capable of oxidizing vascular wall lipids and accelerate atherogenesis in hyperglycemic diabetic patients [31]. The formation of α-dicarbonyl compounds is known to be an essential step for the cross-linking of proteins and subsequent free radical generation. These findings were confirmed recently in a study investigating the reaction of

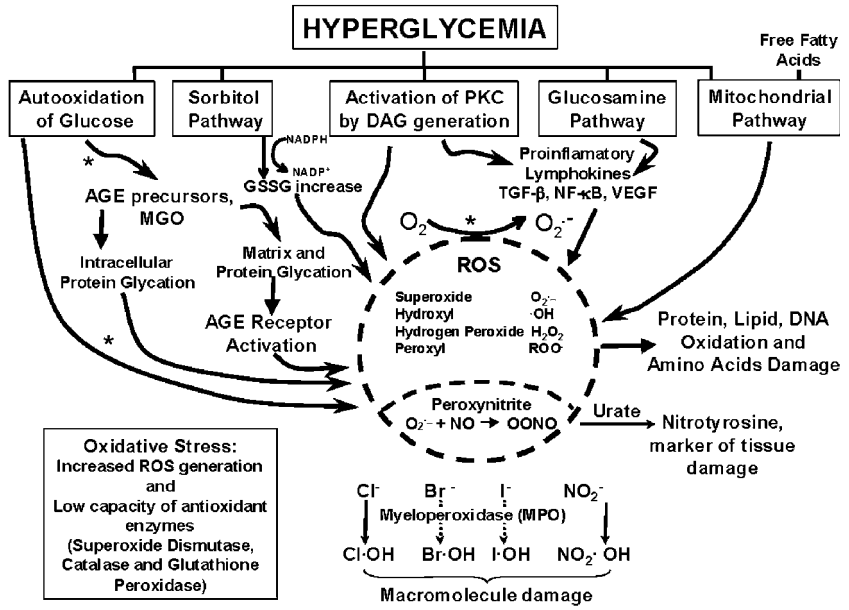


Fig. 1. Pathways for the generation of reactive oxygen species (ROS) and oxidative stress. Asterisks indicate possible sites of inhibition by novel AGE inhibitors.

methylglyoxal (MGO) with the amino acid alanine [27]. In addition to the yellow fluorescent products formed in some glycated protein, three types of free radicals species were also generated. Their structures were identified by EPR spectroscopy and other methods to be cross-linked radical cation, the methylglyoxal radical anion and the superoxide radical anion (which was formed in the presence of oxygen). The authors concluded that α-dicarbonyl cross-linked free amino groups of protein by forming Schiff's bases, which donate electrons to dicarbonyl compounds to form the cross-linked radical cations and methylglyoxal radical anions. Oxygen can accept an electron from the radical anion to generate a superoxide radical anion, which can initiate damaging chain reactions [19,27,30]. These kind of reactions by long-lived glycated protein may contribute to the increased peroxidation of lipids and may also contribute to accelerating oxidative modification of vascular wall lipid in diabetes and atherosclerosis. This finding was also confirmed in a separate study on human dermal fibroblasts exposed to ultraviolet light in the presence of AGE, which resulted in a decrease of cell viability due to superoxide anion radicals generation [28].

In a most recent Editorial Commentary, Vincent Monnier [32] has focused on transition metal redox and protein oxidation in diabetes and oxidative damage in atherosclerosis. Based on several studies reported recently [30,33–37], Monnier has concluded that metal-catalyzed glucose autooxidation and oxidation of glycated residues are potent sources of free radicals and may be the important culprits in tissue damage. In vitro, exposing protein to high levels of glucose causes oxidative protein fragmentation and damage to amino acid residues, with the accumulation of methionine sulfoxide, *o*-tyrosine, *m*-tyrosine, dityrosine, and 3-nitrotyrosine. Many of the glucose associated oxidative modifications have been attributed to Fenton chemistry carried out by transition metals like copper (Cu) and iron (Fe), which are normally present in buffers. In an in vivo study, Pennathur et al. [33] have examined the oxidative chemistry occurring in early atherosclerosis in *Cynomologus* monkeys after six months of streptozotocin (STZ) induced diabetes. They investigated a protein-rich extract from thoracic aorta of diabetic monkeys and found high levels of *o*- and *m*-tyrosine, *o*,*o*′-dityrosine, and 3-nitrotyrosine as markers of damage from hydroxyl radicals that was tightly correlated with levels of glycated hemoglobin in these animals. The strong relationship observed between glycated hemoglobin and hydroxyl radical damage suggested a concomitant process in which CML originates from Amadori products through hydroxyl radical-mediated oxidation [33].

The sequence of events leading to hydroxyl radicals-mediated protein damage in early atherosclerosis in diabetes proposed by Monnier [32] and Saxena [34] is as follows: increased glycation and formation of redox-active center due to the formation of CML and CEL, which can bind redox-active copper and perhaps iron, appear to remain redox-active even when bound to CML/CEL (glycochelates) [35]. Amadori products and ceruloplasmin are also expected to be potent precursors of oxidative damage. CML/CEL metal–protein complex (glycochelates) in the presence of H_2O_2 generates hydroxyl radical ·OH, which reacts with proteins' amino acid residues to form *o*-tyrosine, *m*-tyrosine, and *d*-tyrosine, markers of oxidative stress. The exact source of the transition metals in the above process is not known. Possibilities include the transfer of loosely bound metals to CML/CEL-rich proteins, which could result from glycation of superoxide dismutase (SOD), ceruloplasmin, or ferritin [32].

Glycation

Glycation is a spontaneous non-enzymatic amino-carbonyl reaction between reducing sugars and long-lived proteins and lipids that are a major form of chemical modifications of biomolecules that compromise their function. These chemical damages are detectable in the form of advanced glycation and lipoxidation endproducts (AGE, ALE); amino acids modified by ROS, chlorine, and nitrogen; and racemized amino acids [38]. Glycation is a major source of ROS and reactive α-dicarbonyl intermediates that are generated by both oxidative (glycoxidative) and non-oxidative pathways of glycation. (For a detailed review of glycation pathways, and a list of known AGE, refer to Rahbar and Figarola 2002 [39]).

The toxic effects of AGE (both endogenous and exogenous) result from structural and functional alterations in plasma and extracellular matrix (ECM) proteins, in particular, from crosslinking of proteins and interaction of AGE with their receptors and/or binding proteins, which leads to enhanced formation of reactive oxygen species with subsequent activation of nuclear factor-κB and release of pro-inflammatory cytokines, growth factors, and adhesion molecules [14,40–42]. AGE accumulation in collagen, a long-lived structural protein in the extracellular matrix region of the kidney, is thought to effect changes in elasticity, ionic charge, thickness, and turnover of basement membrane components [43].

Immunohistochemical studies using anti-AGE antibodies have revealed the presence of AGE-modified proteins in several tissues under pathological conditions, including the kidneys of patients with diabetic nephropathy [44], chronic renal failure [45], atherosclerotic lesions of arterial walls [6,46], and amyloid fibroids in hemodialysis-related amyloidosis [47,48], suggesting the potential involvement of AGE-modification in the pathogenesis of age-related disorders [49,50].

Carbonyl stress

Carbonyl stress (Fig. 2) is an imbalance of reactive carbonyl species (RCS) production and carbonyl scavening mechanisms that originate from a multitude of mechanistically related pathways, like glycation [10,51,52], autooxidation of sugars [53], amino acid metabolism [51], lipid peroxidation [54], and UV damage [49]. An important step in the glycation reactions is the generation of reactive intermediate products in the course of all stages and pathways of glycation. These compounds are known as α-dicarbonyls (α-oxaloaldehyde) and include such products as 3-deoxyglucosone (3-DG), glyoxal (GO) and methylgoxal (MGO). 3-DG is formed by non-oxidative rearrangement and hydrolysis of Amadori product and by fructose-3-phosphate, an intermediate of polyol pathway. 3-DG rapidly reacts with protein amino groups to form AGE such as imidazolone, pyrraline, and CML [10,54,55]. MGO may be produced by non-enzymatic pathways from spontaenous decomposition of triose phosphates, autoxidation of carbohydrates and glucose degradation, and also by several minor metabolic pathways, including the Maillard reaction and lipid peroxidation [10,56–58]. In addition to reaction with arginine residues to form imidazolone adducts, MGO reacts with lysine residues in protein to form CEL and the imidazolium crosslink, methylglyoxal-lysine dimer (MOLD) [58,59]. It is noteworthy that MGO is produced by most glucose metabolizing cells. GO is formed from several reactions such as oxidative fragmentation of Schiff's base (Namiki pathway), glucose autoxidation and degradation, lipid peroxidation, and fructose-phosphate fragmentation [10,54,60]. Recently, a new pathway for generation of glyoxal has been described through peroxynitrite ($ONOO^-$)-mediated oxidation of glucose [61]. Production of GO in vivo under physiological conditions can yield a variety of AGE such as CML, pentosidine, GOLD, GOLA, and other non-fluorescent AGE [54,62–65].

Under normal conditions, very little flux of α-oxoaldehyde and fructosamine formation proceeds to form AGE because there are major alternative metabolic fates of these AGE precursors. α-Oxoaldehydes are metabolized and inactivated by enzymatic conversion to the corresponding aldonic acids, catalyzed by the glutathione-dependent glyoxalase system [66]. 3-DG is metabolized to 3-deoxyfructose, catalyzed by NADPH-dependent 3-DG reductase, while fructosamine is degraded to Schiff's base adduct by reversal of the Amadori rearrangement, and to fructosamine-3-phosphate by the ATP-dependent 3-phosphokinase [67]. Advanced glycation by α-oxoaldehydes is also decreased by reversible binding of α-oxoaldehydes to cysteinyl thiols, forming hemiothioacetal adducts [67]. The formation of AGE is increased when the concentrations of oxoaldehydes and fructosamine are increased. This may arise as a consequence of increased rates of formation and/or decreased rates of metabolism of α-oxoaldehydes and fructosamine [67,68]. Hyperglycemia, accumulation of triosephosphates and ketone bodies, lipid peroxidation, and oxidative stress may all increase the formation of AGE [10,67,68].

The accumulation of MGO and other reactive dicarbonyls from both glycoxidation and lipoxidation

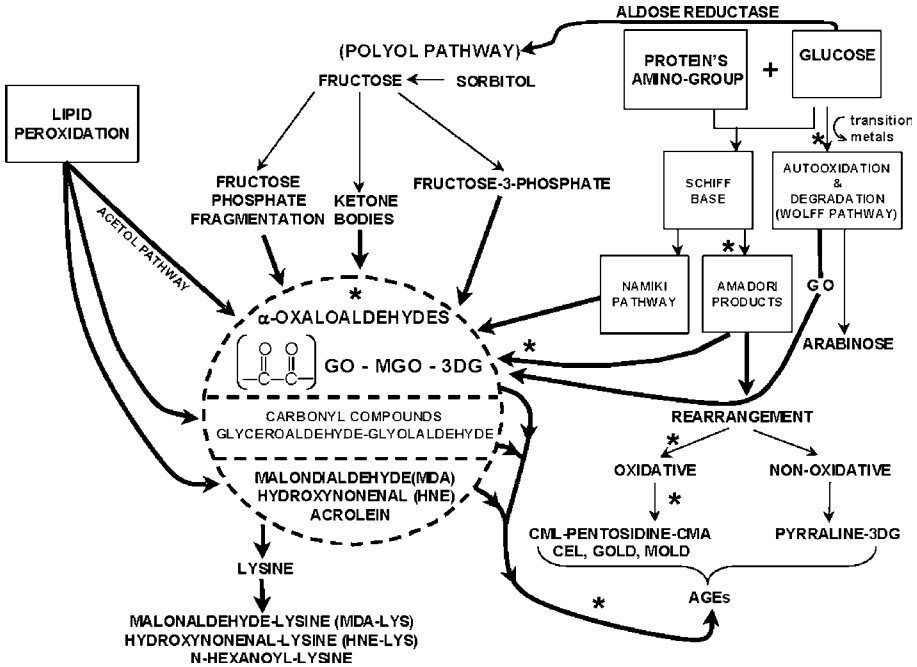


Fig. 2. Sources of reactive carbonyl species (RCS) and carbonyl stress. Asterisks indicate possible sites of inhibition by novel AGE inhibitors.

sources is called *carbonyl stress* [26,51,65,69,70] (Fig. 2), a phenomenon accelerated in diabetes and in uremia. This carbonyl stress hypothesis emphasizes the role of RCS, derived from various sources, in the induction of pathogenic protein, lipid, and DNA modifications. As stated, α-oxaloaldehydes are formed in early glycation from the degradation of glucose, Schiff's base adducts and Amadori products are important precursors of AGE. The formation of these reactive carbonyl species is dependent on the availability of trace metal ions [10]. Chelation of trace metal ions by DTPA markedly inhibits their formation in the cascades of a number of their catalytic pathways [10]. Thus, therapeutic approaches based on chelation of metal ions such as copper or iron may have dual inhibitory function on redox activity, suppressing ROS production as well as indirect inhibition of α-oxaloaldehyde generation and carbonyl stress.

Recent studies by Michael Brownlee et al. [14,71,72] have emphasized on increased production of ROS in the pathologies of diabetic complications. Based on their studies on increased ROS production inside cultured bovine aortic endothelial cells in hyperglycemic conditions, these authors showed that ROS may activate aldose reductase, induce diacylglycerol, activate PKC, induce AGE formation, and activate nuclear factor-κB (NF-κB). Furthermore, they reported that normalizing mitochondrial superoxide production using an inhibitor of electron transport chain complex II, by uncoupling protein-1 (UPC-1), and by manganese superoxide dismutase (MnSOD), prevented glucose-induced activation of PKC, formation of AGE, sorbitol accumulation, and NF-κB activation. Their data demonstrate that a single unifying mechanism of induction increased the production of ROS and serves as a causal link between hyperglycemic and each of the three major pathways responsible for diabetic damage namely: glucose-induced activation of protein kinase C (PKC) isoforms; increased formation of glucose-derived AGEs; and increased glucose flux through the aldose reductase pathway.

Recently, Baynes [26] addressed the role of oxidative stress among other hypothesis in diabetic complications. He believed that because oxidative stress and the AGE hypothesis are inextricably intertwined "the question is not so much whether oxidative stress is increased in diabetes, since oxidative stress and oxidative damage to tissues are common end points of chronic diseases, such as atherosclerosis, diabetes, and rheumatoid arthritis, but whether oxidative stress has a primary roll in pathogenesis of diabetic complications. At issue is whether oxidative stress occurs at an early stage in diabetes, proceeding the appearance of complications, or whether it is merely a common consequence of the tissue damage, reflecting the presence of complications" [65].

The increase in glycoxidation and lipoxidation products in plasma and tissue proteins suggests that oxidative stress is increased in diabetes. However, some of these products, such as 3-DG adducts to lysine and arginine resides, are formed independent of oxidative chemistry. Furthermore, based on studies reported by Wells-Knecht et al. [73], age-adjusted levels of oxidized amino acids (methionine sulfoxide and *o*-tyrosine), which are direct indicators of oxidative stress, are not increased in skin collagen in diabetes. In his concluding remarks, Baynes [25] proposed that the increased chemical modification of proteins by carbohydrates and lipids in diabetes is the result of overload on metabolic pathways involved in detoxification of reactive carbonyl species, leading to a general increased in steady-state levels of reactive carbonyl compounds formed by both oxidative and non-oxidative reactions and then, at a late stage, oxidative stress and tissue damage happens.

Natural defense mechanism against AGE and cell signaling pathways

Nature has devised several humoral and cellular defense mechanisms to protect tissues from deleterious effects of carbonyl stress and accumulation of AGE. These include the glyoxylase system (I and II) and aldose reductase that catalyze the deglycation of methylglyoxal (MG), the most common reactive intermediates of AGE to D-lactate [66,74]. Additionally, a novel class of enzymes found in *Aspergillus* called amadoriases was found to catalyze the deglycation of Amadori products [75]. Most recently, human fructosamine-3-kinase (FN3K) has been identified which phosphorylates fructoselysine (F1) residues on glycated proteins to F1-3-phosphate and lead to its spontaneous decomposition, thereby reversing the non-enzymatic glycation process at an early stage [76]. Other enzyme systems involved in the enzymatic regulation of AGE formation include enzymes that metabolize the α-dicarbonyl (α-oxaloaldehydes) such as endogenous enzymes capable of reducing 3-DG. Two of these, NADPH-dependent enzymes are oxoaldehyde reductase and aldose reductase which are able to detoxify reactive dicarbonyl intermediates [77–79]. Gluthathione (GSH) system has a dual function in the cell, as antioxidant and coenzyme; it acts in this capacity when used by GSH peroxidase to reduce peroxides or superoxide, yielding GSSG. However, GSH also has an independent detoxification function in the glyoxylase pathway when it facilitates the rearrangement of dicarbonyl to hydroxyacids such as MGO-lactate [74].

Several AGE receptors have been characterized on the surface membranes of monocytes, macrophage, endothelial, mesangial, and hepatic cells, including macrophage scavenger receptors type I and II, oligosaccharyl transferase-48 (AGE-R1), 80 K-H phosphoprotein (AGE-R2), and galectin-3 (AGE-R3) [80–82],

and RAGE, a member of the immunoglobulin superfamily, which has been found to have a wide distribution in tissues [83,84]. RAGE is a multiligand membrane receptor and is thought to act as a scavenger and mediate intracellular signalling. In vitro studies have shown at AGE–RAGE binding on macrophages and microglia lead to generation of ROS and activation of nuclear transcription factor NF-κB [85–87]. This process involves the activation of the $p21^{ras}$/MAP kinase signaling cascade, which activates NF-κB and generation of AGE–RAGE interaction [88]. Blockade of RAGE, using soluble RAGE, the extracellular ligand-binding domain of the receptor, has been reported to enhance wound closure in genetically diabetic animals by suppressing the levels of some inflammatory cytokines, promoting vascularized granulation of tissues, and increasing the levels of PDGF-B and VEGF [89]. Similarly, treatment of diabetic mice with the soluble RAGE completely suppressed diabetic atherosclerosis in a glycemia- and lipid-independent manner [90].

Dietary antioxidants

A dietary antioxidant can be defined as "*a substance in food that significantly decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans*" [13]. Antioxidant compounds such as vitamin C, vitamin E (α-tocopherol and the carotenoids including α-carotene, β-carotene, β-cryptoxantin, lutein, lycopene, and zeaxanthine) have been shown to have vascular-related effects that prevent or reverse nerve conduction velocity (NCV) deficits in experimental models [91]. They have also shown to reduce in vitro and in vivo protein glycation. For example, treatment of diabetic rats with vitamin E resulted in a decrease of plasma lipid peroxidation [92], and a multi-center double blind study in 328 type 2 diabetic patients showed improvement of some clinical features of neuropathy after 3-week treatment of lipoic acid [93]. In several in vitro experiments under strict antioxidative conditions, using ribose as glycating sugar, we have recently investigated the AGE inhibitory activities of a number of known antioxidant compounds, including 5-aminosalicylic acid (5-ASA) [39], *N*-acetylcysteine, lipoic acid, lipoic acid amide, taurine, para-aminobenzoic acid (PABA), para-aminosalicylic acid, aspirin, benzoic acid, salicylic acid, inositol, and probucol, by several in vitro assay methods. Aspirin, salicylic acid, PABA, and benzoic acid were found to have moderate AGE-inhibitory effects while inositol and probucol were strong AGE-inhibitors [S. Rahbar, unpublished data]. These findings suggest that some of these antioxidants may have some AGE-inhibitory effects in vitro primarily by preventing the autoxidative pathways of AGE formation. Nevertheless, although a growing body of evidence shows that the production of ROS is increased in diabetes, and that diabetic complications is associated with oxidative stress, numerous clinical trials have failed to provide conclusive evidence for the efficacy of antioxidant therapy in diabetic patients [94].

Synthetic and natural AGE-inhibitors

Historically, nearly a century ago, high-dose salicylate treatment was found to reduce the glycosuria in diabetic patients [95]. The amino acid lysine and *N*-acetyllysine also demonstrated beneficial effects in reducing albuminuria in diabetic subjects [96]. However, the first compound which has been extensively studied in vitro and in vivo to be a powerful inhibitor of AGE formation is aminoguanidine [97]. In the original study, aminoguanidine inhibited the cross-linking and fluorescence of aortic collagen in diabetic rats. Later studies by several investigators demonstrated that aminoguanidine retarded the development of diabetic complications including nephropathy, neuropathy, and vasculopathy [98]. However, AG had no effects on hyperglycemia. Aminoguanidine, a hydrazine-like small molecule, is a nucleophilic compound that traps reactive carbonyl intermediates such as MGO, GO, and 3-DG [99]. It is known to inhibit CML and CEL formation, cross-linking, and fluorescence in skin collagen of diabetic rats, and significantly retard the development of diabetic nephropathy. AG also caused correction of hyper-cholesterolemia and hypertriglyceridemia in the diabetic rats, consistent with effects of AG on dyslipidemia in humans [98]. AG was also found to be a potent inhibitor of nitric oxide synthase (NOS) [100]. Among the side effects reported in patients treated with aminoguanidine was pernicious-like anemia, and of greater concern for the development of anti-nuclear antibodies (ANF) in high-dose AG therapy [101,102]. Also, higher rates of pancreatic and renal-neoplastic tumors were reported in diabetic rats treated with AG [77]. Unfortunately, the clinical phases in the aminoguanidine treatment of patients with type-1 diabetes were ended due to serious complications in those patients. Most recently, ALT-946, *N*-(2-acetamidoethyl) hydrazinecarboximidamide hydrochloride, a new inhibitor of AGE having hydrazine group in its structure, was found to have minimal inhibitory effects on NOS as compared with AG in experimental diabetic nephropathy [103].

We recently evaluated the in vitro effects of three existing drugs, metformin, pioglitazone, and pentoxyfylline on AGE formation and demonstrated that all of these three drugs are potent inhibitors of glycation [104]. Metformin (dimethylbiguanide) (Glucophage) is an oral antihyperglycemic drug used in the management of type 2 diabetes and has some structural similarities to aminoguanidine. Beisswenger et al. [105] observed that type

2 diabetic patients have increased MGO concentrations in blood, which were significantly reduced by high dose metformin treatment. The mechanism of reduction of circulatory MGO by metformin is suggested to be the trapping of MGO and other dicarbonyls [106,107]. The data we presented in our report indicate that in fact metformin is a potent inhibitor of glycation, possibly by its interaction with dicarbonyl compounds generated during the glycation process. Using new assay methods specific for the early (Amadori) and late stages of glycation (post-Amadori) [108], we have shown that metformin is a multistage inhibitor of glycation with greater effects in the post-Amadori stages [104]. While AG has demonstrated to be an inhibitor of Amadori stage, it shows little post-Amadori inhibitory effects [108]. The mechanisms of inhibitory effects of pioglitazone and pentoxyfylline remain unknown to us, although oral antidiabetic agents like troglitazone have been shown to have antioxidant activity [109].

Other compounds having AGE inhibitory activity include D-penicillamine and desferoxamine, perhaps due to antioxidant properties [110,111]. Thiamine pyrophosphate and pyridoxamine were shown to be effective inhibitors of AGE formation of post-Amadori type [112]. Anti-inflammatory compounds such as acetylsalicylic acid, ibuprofen, and indomethacin were reported to be inhibitors of glycation [113–115], perhaps by preventing the oxidative stress associated with the formation of AGE. Diclofenac (Voltran), a non-steroidal anti-inflammatory drug, was demonstrated in vitro to be an inhibitor of AGE, presumably by a non-covalent interaction of the drug with serum protein [116]. Inositol was reported to be an inhibitor of glycation of lens crystalline protein in vitro by its quenching action on reactive oxygen and the formation of glucosyl–inositol complex [117].

Benfotiamine, a lipid soluble compound, was found to be a potent inhibitor of glycation [118]. This drug was recently shown to block the three major pathways of hyperglycemic damage and was successful in preventing diabetic retinopathy in rats [119]. Carnosine (β-alanyl-histidine) is a natural dipeptide that is widely distributed in mammalian tissues, including muscles and brain in high concentrations. Various biological functions of carnosine including its role as an antioxidant, metal-chelator, SOD mimetic, and free radicals have made carnosine an interesting AGE inhibitor for in vitro and in vivo studies [120,121]. Tenilsetam, (+)-3-(2-thienyl)-2-piperazine, a cognition-enhancing and possible anti-dementia drug was also shown to be an effective AGE inhibitor. Although the mechanism of its action is yet to be understood, it reacts with sugars and glycated proteins and acts as an inhibitor of protein cross-linking [122].

There are reports of some natural substances isolated from plants with AGE-inhibitory effects. One such compound is resveratrol (3,4,5-trihydroxystilbene), a natural phytoesterogen found in grapes [123]. Resveratrol has been shown to inhibit AGE-induced proliferation and collagen synthesis activity in vascular smooth muscle. Another natural compound, curcumin, an active principle isolated from turmeric (*Curcuma longa*), has been known for its anti-oxidant and anti-inflammatory properties. Studies have revealed curcumin to be a potent inhibitor of AGE formation and cross-linking of collagen in diabetic rats [124].

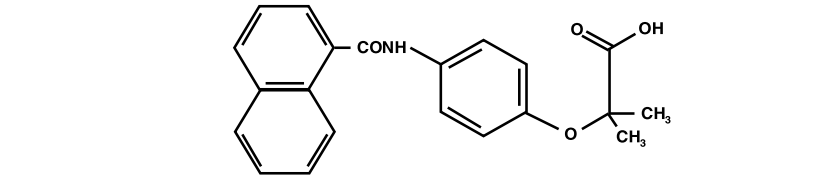
A large number of synthetic compounds have been designed and synthesized as AGE inhibitors. ALT-946, an inhibitor of AGE with minimal inhibitory effect on nitric oxide synthase, was developed by Alteon and showed renoprotective effects in diabetic rats [103]. Diaminophenazine (2,3 DAP), a novel inhibitor of AGE formation, inhibited mesenteric vascular hypertrophy in experimental diabetes in rats [125]. Synthetic compounds with thiazolidinedione structure have been found recently to be effective AGE inhibitors. OPB-9195 [(±)-2-isopropylidenhydrazono-4-oxo-thiazolidin-5-ylacetalinide] has been reported to be a potent inhibitor of AGE and advance lipoxidation endproducts [126]. Pioglitazone, another member of the thiazolidinedione family, was reported before by our laboratory as a powerful AGE inhibitor [104]. The mechanism of action may be assumed to be the trapping of dicarbonyl groups by the hydrazine nitrogen groups of these compounds, as well as metal-chelation activities.

Novel AGE-inhibitors

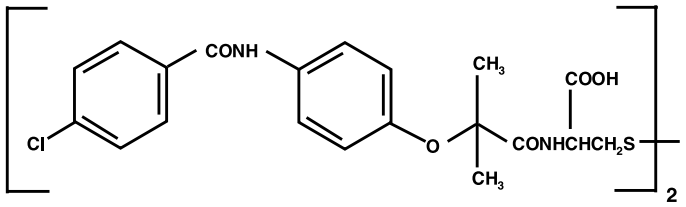
In the past seven years, our laboratory in collaboration with Dr. I. Lalezari, an organic chemist at Chemiphar, New York, has been involved in the design and synthesis of compounds with possible AGE inhibitory effects. These aromatic compounds, mostly derivatives of aryl (and heterocyclic) ureido, and aryl (and heterocyclic) carboxaminido phenoxy isobutyric acids, were screened and evaluated using several well-established in vitro assay methods. Drug candidates examined in this screen were derivatized further in the hope of finding more effective compounds, and small libraries (115 compounds) based on the initial screening and structure-function relationship studies were identified. By starting with known AGE inhibitors, we were able to improve upon existing compounds and developed 31 novel compounds (out of 115) with AGE-inhibitory activities (16 weak, 5 medium, and 10 with the highest activity) [39,127,128]. A list of some of these novel inhibitors and their chemical structures are presented in Table 1 and Fig. 3. Most of our compounds are multi-stage glycation inhibitors with the highest post-Amadori activities (Fig. 4). Some of these compounds inhibited AGE-protein crosslinking, including AGE-collagen crosslinking derived from the reaction of bovine serum albumin with

Table 1
Novel compounds as inhibitors and breakers of AGE

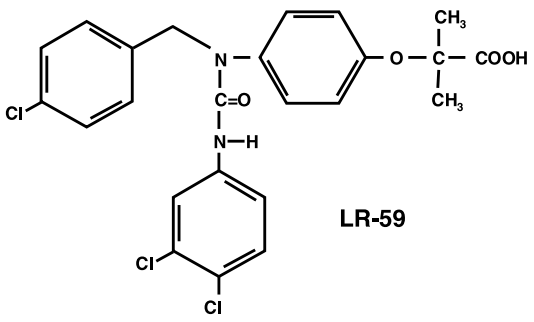
A: Novel AGE-Inhibitors
LR-9, 4-(2-naphtylcarboxamido) phenoxyisobutyric acid
LR-20, L-bis-4[-(4-chlorobenzamidophenoxyisobutyryl) cystine
LR-23, 4-(3,5-dichlorophenylureido-phenoxyisobutyryl-1-amidocyclohexane-1-carboxylic acid
LR-33, 4-(2-chloro-4-nitrophenylureido) phenoxyisobutyric acid
LR-41, 4-(3-chloro-4-fluorophenylureido) phenoxyisobutyric acid
LR-59, 4-[(3,4-dicholorophenylmethyl) 2-chlorophenylureido] phenoxyisobutyric acid
LR-62, 4-(2,4-dichlorophenacylamino) phenoxyisobutyric acid
LR-74, 2-(8-quinolinoxy) propionic acid
LR-90, Methylene bis [4,4'-(2-chlorophenylureidophenoxyisobutyric acid)]
LR-102, 1,4-benzene-bis[4-methyleneaminophenoxyisobutyric acid]
B: Novel AGE-Breakers
LR-20, L-bis-4[-(4-chlorobenzamidophenoxyisobutyryl) cystine
LR-23, 4-(3,5-dichlorophenylureido)-phenoxyisobutyryl-l-amidocyclohexane-1-carboxylic acid
LR-99, 4-[(3,5-dichlorophenylureidophenoxyisobutyryl]-4-aminobenzoic acid)]
LR-102, 1,4-benzene-bis [4-methyleneaminophenoxyisobutyric acid].
SMR-5, 5-aminosalicylic acid (5-ASA)
SMR-12, dimethylbiguanide (metformin)



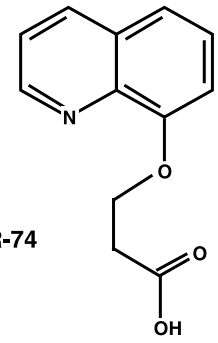
LR-9



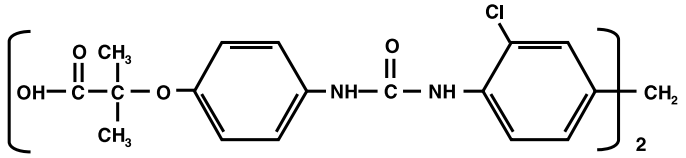
LR-20



LR-59



LR-74



LR-90

Fig. 3. Chemical structures of some of the novel AGE inhibitor compounds.

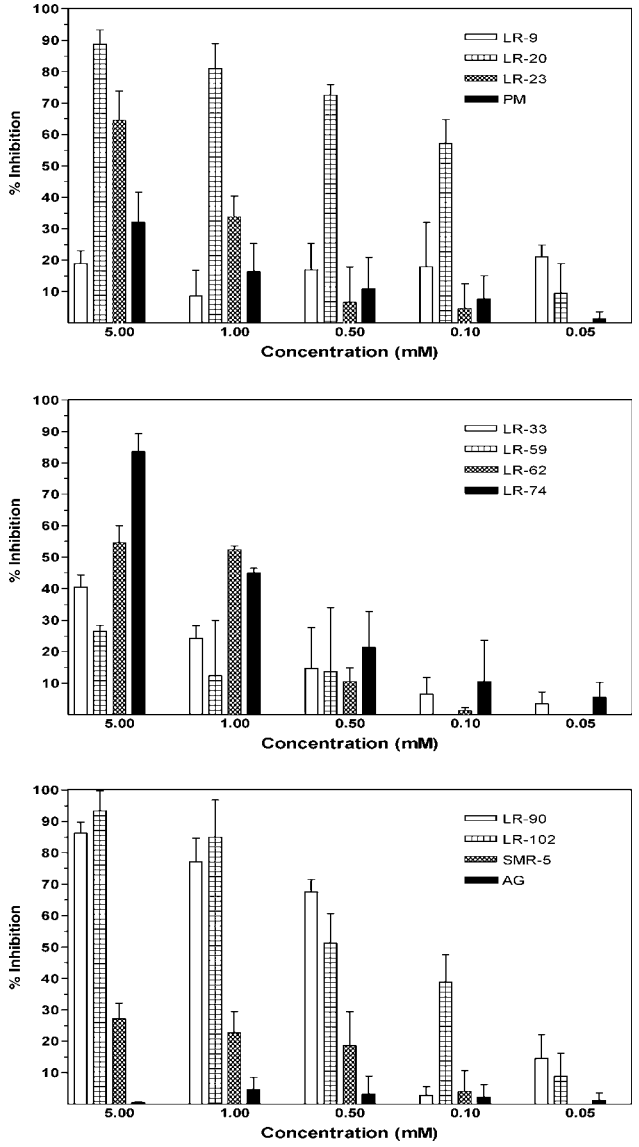


Fig. 4. Inhibition of post-Amadori AGE formation by novel compounds. BSA (10 mg/ml) was incubated with 0.5 M ribose in 0.4 M phosphate buffer, pH 7.5, containing 0.02% sodium azide for 24 h at 37 °C, followed by extensive dialysis for 24 h at 4 °C to remove excess and reversibly bound ribose. AGE formation was then initiated by incubating 0.1 mg/ml (or 1:100 dilution in phosphate buffer) of the glycated protein in the absence and presence of various concentrationsof the compounds at 37 °C for 7 days. Aliquots from each sample were taken and diluted with 0.1 M sodium carbonate buffer to 50 μg/ml. Then, 50 μl of the diluted sample was added to wells of a 96-well polystyrene plate (1.0 μg/well) and incubated overnight at room temperature. The amount of post-Amadori products in each treatment was then quantified by ELISA using polyclonal anti-AGE-RNAse antibodies. Percent of post-Amadori inhibition was calculated as: 100* (mean OD wells without compound-mean OD wells with compound/mean OD wells without compound). Aminoguanidine and pyridoxamine were also analyzed as controls.

glucose (Fig. 5), as well as fructose-derived AGE-lysozyme crosslinking (data not shown). In addition, some of these compounds inhibited MGO-derived protein crosslinking (Munch and Rahbar, unpublished data).

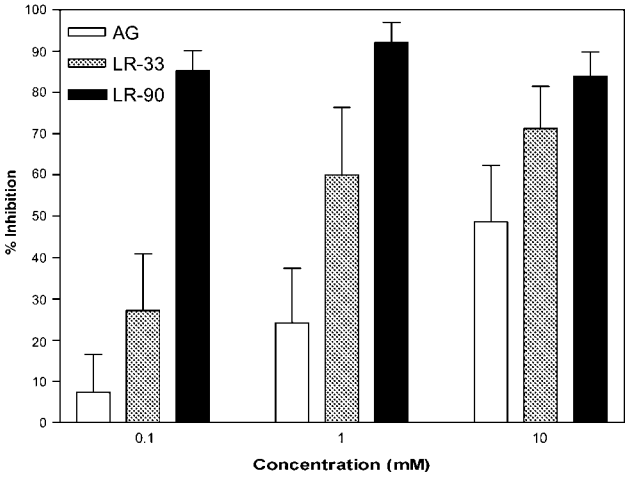


Fig. 5. Inhibition of AGE-BSA crosslinking to immobilized collagen by novel compounds. AGE-BSA was crosslinked to rat tail tendon collagen coated wells in the presence and absence of novel compounds and aminoguanidine. Collagen–AGE-BSA complex was measured using rabbit-anti-AGE-RNase polyclonal antibodies. Crosslink inhibition was calculated using the formula: 100* (mean OD control wells-mean OD test wells/mean OD control wells).

In vivo investigation on LR-90, one of the novel AGE inhibitors, showed promising results in preventing the progression of diabetic nephropathy in diabetic rats. We observed that LR-90 treatment of 50 mg/l in drinking water of diabetic rats for 32 weeks markedly prevented the increase in albumin and creatinine excretions in urine without any effects on glycemic control [129]. AGE concentrations in serum and accumulation in kidney glomeruli were also substantially decreased by the drug (Fig. 6). LR-90 treatment also decreased protein crosslinking in collagen tissues, and prevented basement membrane thickening, mesangial expansion, and collagen deposition in the tubulointerstitium, indicating that this drug effectively inhibited tissue damage associated with AGE formation and AGE-protein crosslinking. Moreover, the degree of oxidative damage to kidney glomeruli and tubules, as measured by levels of nitrotyrosine, a marker for protein oxidation, was also attenuated by the drug [129]. These findings support earlier in vivo studies on several AGE inhibitors that compounds that can inhibit AGE formation can confer renoprotective effects. We are currently testing other novel AGE inhibitors we have identified in vitro for similar effects on diabetic rats.

Possible mode of action of novel compounds

Although aldose and ketose sugars were originally thought to be the sole precursors of AGE, current research indicates that RCS generated from carbohydrate, lipid, and amino acid metabolisms such as MGO, GO, GLA, dehydroascorbate, 3-deoxyglucosone, and malondialdehyde are even more reactive and are potent

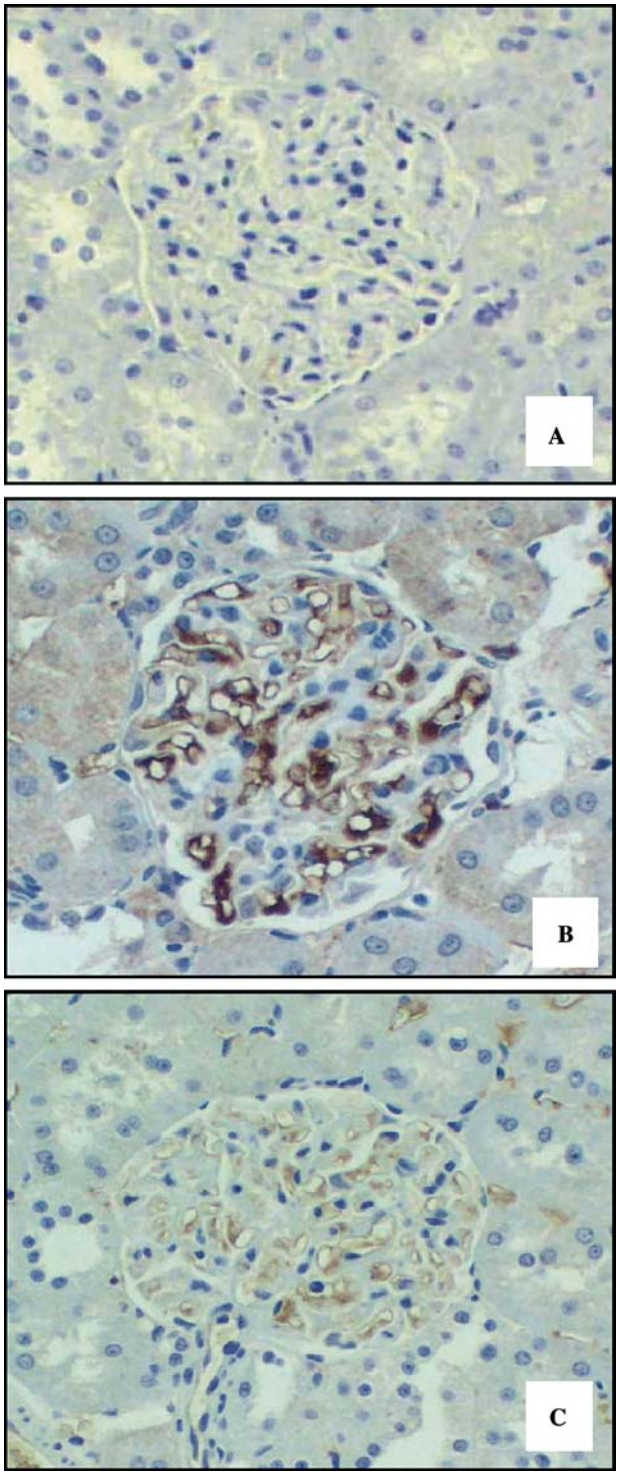


Fig. 6. Effect of LR-90, a novel AGE inhibitor, on the levels of AGE in renal glomeruli and mesangial matrix. Diabetic male Sprague–Dawley rats were given LR-90 at 50 mg/L in drinking water for 32 weeks. At the end of the study, kidneys from these rats, together with age and weight-matched non-diabetic and diabetic control rats, were isolated and fixed with formalin and sections (2 μm thick) were cut and mounted on slides. The slides were deparaffinized and blocked, and AGE levels were detected immunologically using anti-AGE monoclonal (6D12) antibodies specific for CML. (A) non-diabetic; (B), diabetic; and (C) diabetic + LR-90. Results shown are representative images from different kidney samples from each group.

precursors of AGE formation and protein crosslinking [10,25,51,129,130] (Fig. 2). Known AGE inhibitors with renoprotective effects such AG, PM, and OPB-9195 are thought to prevent AGE accumulation by interacting with these highly reactive RCS and acting as carbonyl traps, thereby limiting oxidative damage to tissues. AG reacts with α-dicarbonyls GO, MGO, and 3-deoxyglucosone to form triazine derivatives [99,131], while PM was recently shown to interact and form adducts with GO, GLA, and MGO [132,133]. It has also been suggested that the chelating activity of AGE inhibitors and AGE breakers at therapeutic concentrations may contribute to their inhibition of AGE formation and protection against development of diabetic complications [134].

The mechanism(s) of action of some of our novel AGE inhibitors is still unclear, but our in vitro studies indicate that most of them are potent chelators of Cu^{2+} and inhibit oxidation of ascorbic acid to dehydroascorbate (Fig. 7 and Table 2). Most of the novel LR compounds analyzed show greater chelating activities than pyridoxamine and aminoguanidine. LR-9, LR-59, LR-74, and LR-90 are potent chelators, with IC_{50}'s of ~50 to 275 μM. Spectroscopic studies confirm the formation of inhibitor-copper complexes [S. Rahbar, unpublished data]. Recent studies [135] suggest that metal-catalyzed oxidation plays a critical role in glucose-induced modifications of the collagen. Transition metals like copper ions can catalyze both glycation (covalent binding of glucose to collagen) and glycoxidation (oxidation of glycated collagen) in a concentration-dependent manner [135]. Of major interest along this context is the suggestion that CML and possibly other glycated proteins can complex with redox active metals, creating a protein–metal redox active center that could generate free radicals and initiate hydroxyl-radical-mediated tissue damage [32,34–36]. By gradually removing free metal ions from tissues and plasma for excretion in urine, these novel compounds could decrease the overall metal-catalyzed oxidative damage to proteins in vivo. This was clearly evident with the results obtained in our in vivo studies with LR-90 where we observed that the drug prevented CML-AGE accumulation in kidneys, reduced AGE-collagen cross-linking in tissues, and prevented oxidative damage to renal glomeruli and tubules [129]. Preliminary results obtained with LR-74 and LR-90, two of the more potent metal chelators, revealed that at least in vitro, these compounds can suppress hydroxyl radical production from sugar autoxidation and initial glycation reactions, probably by sequestering metals required for hydroxyl radical formation from H_2O_2 (J.L. Figarola and S. Rahbar, unpublished observations).

We also observed that some of these compounds could directly interact with several RCS such as GO, MGO, and GLA in vitro (Fig. 8). These interactions

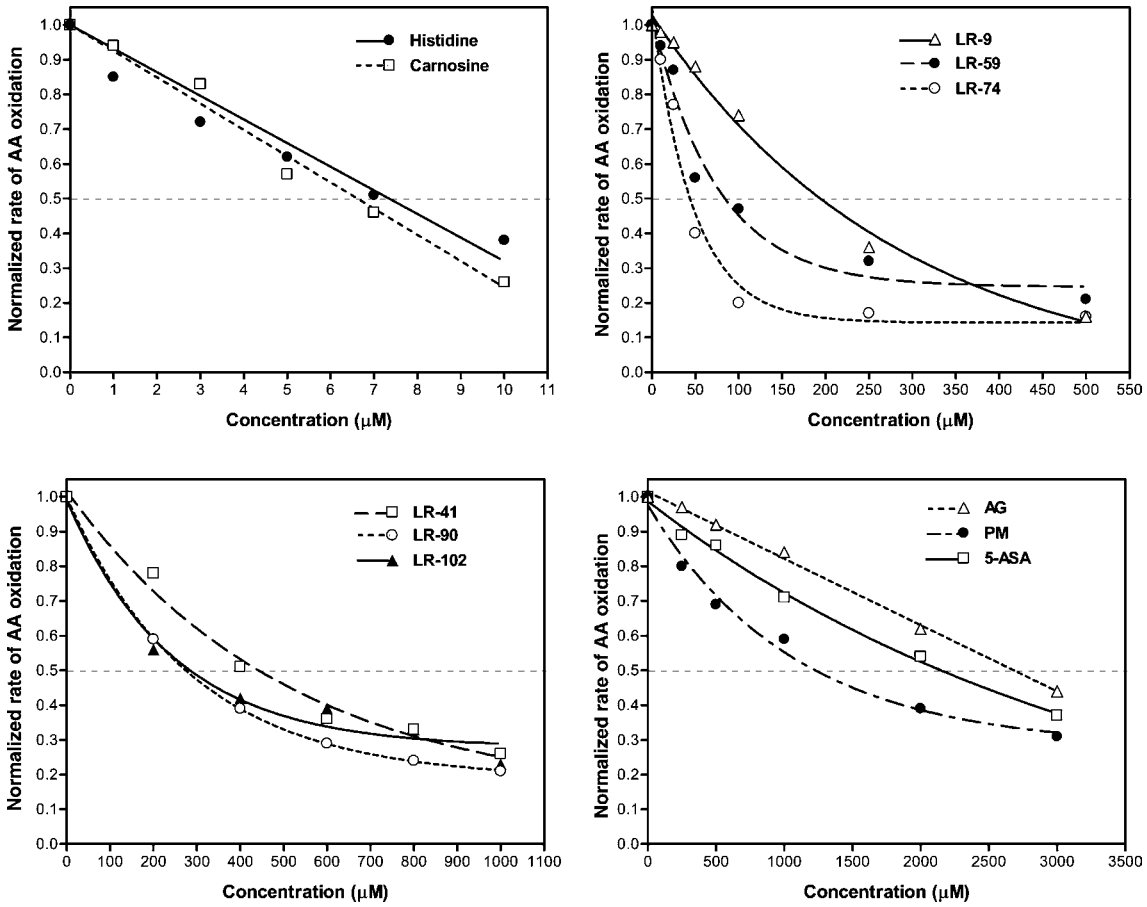


Fig. 7. Effects of biological amines and various AGE formation inhibitors on the kinetics of copper (II)—catalyzed oxidation of ascorbic acid. The kinetics of copper-catalyzed oxidation of ascorbic acid (AA) were measured by RP-HPLC from reaction mixtures of 500 μM AA, 500 nM $CuCl_2$, and various concentrations of inhibitor compounds in 20 mM chelex-treated phosphate buffer (126). Dashed horizontal line indicates 50% loss of AA.

Table 2
Estimated IC_{50} for inhibition of copper-catalyzed oxidation of ascorbic acid by various amines, antioxidants and novel AGE inhibitor compounds

Compound	IC_{50} (μM)
AG	2750
Histidine	8
Carnosine	7
PM	1250
Pioglitazone	300
α-Lipoic acid	125
SMR-5 (5-ASA)	2200
Metformin	>3000
LR-9	200
LR-20	90
LR-23	>3000
LR-33	>3000
LR-41	450
LR-59	90
LR-62	>3000
LR-74	50
LR-90	275
LR-102	300

could inhibit protein modifications and subsequent free radical generation caused by these RCS. Although we have not yet identified the products/adducts between these compounds and the various RCS, we have observed that some novel compounds also prevents the formation of non-CML AGE and protein crosslinks derived from both RCS-protein interactions (Fig. 9 and S. Rahbar, unpublished data). In addition, LR-90 was able to reduce superoxide generation resulting from the interaction of MGO with *N*-α-acetyl-L-lysine in the absence of transition metals (J. Figarola, unpublished observations). Such inhibition of superoxide formation may explain why our in vivo results showed that this compound decreased nitrotyrosine levels in the renal tubules of diabetic rats, preventing further oxidative damage. A recent study [136] revealed that the increase in CML-AGE and nitrotyrosine staining in rats with diabetic nephropathy can be attenuated by ramipril and aminoguanidine, indicating that ACE inhibition and blockage of AGE formation could involve common pathways such as ROS formation.

Using various assay methods to screen the in vitro efficacy of these novel compounds, we found little

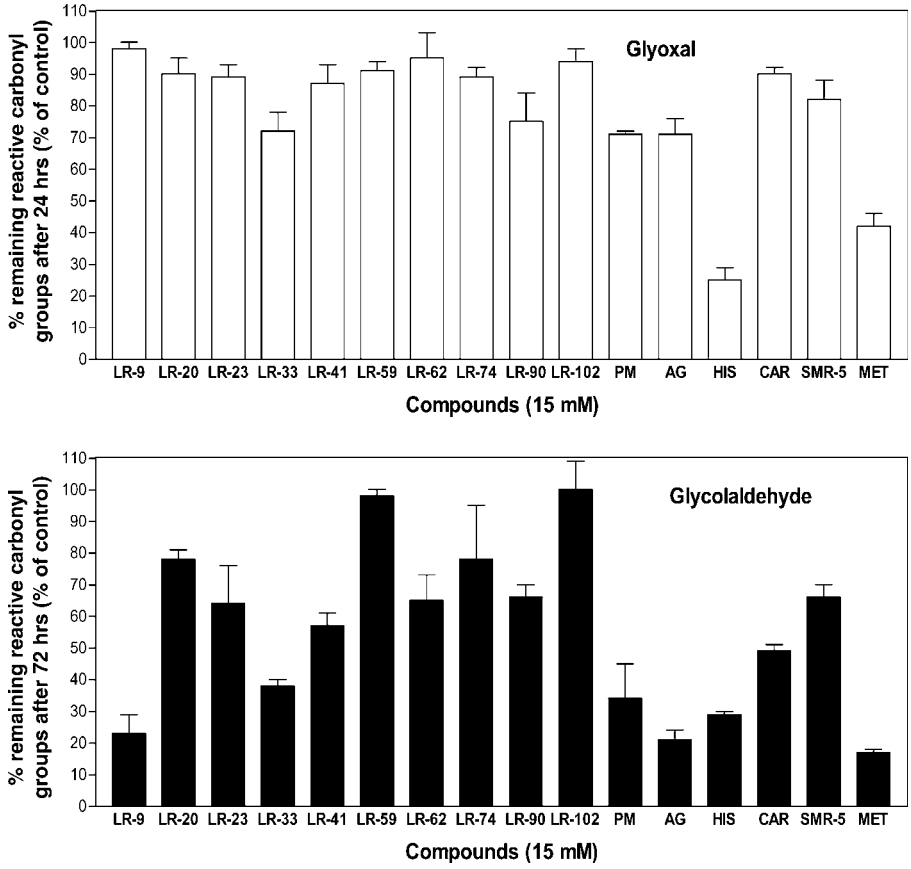


Fig. 8. Reaction of various AGE inhibitor compounds with reactive carbonyl groups. Triplicate samples containing 10 mM glyoxal or 10 mM glycolaldehyde were incubated with 15 mM of inhibitor compound at 37 °C in 200 mM sodium phosphate buffer, pH 7.5, containing 0.2 g/L sodium azide. Incubation time was 24 h for GO and 72 h for GLA (124). Parallel experiments with carbonyl compounds incubated under the same conditions but without the inhibitor compound were used as reference for calculations of the relative amount of reactive carbonyl groups remaining after the incubation period. The loss of carbonyl groups in the course of incubation was measured spectrophotometrically using either Girard's T reagent (for GO), or DNPH (for GLA). AG, aminoguanidine; CAR, carnosine; HIS, histidine; MET, metformin; PM, pyridoxamine; and SMR-5, 5-aminosalicylic acid.

correlation between the metal chelation properties and carbonyl scavenging activities of these compounds, as well as how they inhibit post-Amadori AGE formation and AGE-protein crosslinking in vitro. For example, we found LR-9 and the biological amines carnosine and histidine as potent metal chelators and excellent carbonyl scavengers, yet these compounds are weak post-Amadori AGE inhibitors. In contrast, LR-20, LR-74, and LR-102 showed very high post-Amadori inhibitory and metal chelation properties, but with low to moderate carbonyl scavenger properties (Table 2, Figs. 7 and 8). Furthermore, metformin readily interacts with RCS such as MGO, but it does not chelate metal ions. LR-90, the compound that showed in vivo efficacy in preventing diabetic nephropathy, was a potent chelator and inhibitor of post-Amadori AGE; it also has a moderate carbonyl scavenger activity (Table 2, Figs. 7 and 8). However, all of these compounds effectively inhibited AGE-lysozyme protein crosslinking (Figarola and Rahbar, unpublished observations). Since AGE can be formed by multiple pathways involving both oxidative and non-oxidative processes, compounds that can trap RCS and also those that can sequester transition metals may be good candidate AGE inhibitors in vivo.

AGE-protein crosslink breakers

Recently, novel compounds such as phenacylthiazolium bromide (PTB), and its derivative ALT 711, which are able to selectively cleave and break the established AGE-protein cross-links in vitro and in vivo were reported [137,138]. Using modified screening methods, our laboratory has identified some potential AGE breakers belonging to the same group of AGE inhibitors (Table 1). For example, three of the novel AGE inhibitors (LR-20, LR-23, and LR-102), together with 5-ASA and metformin, showed moderate breaking properties on AGE-protein crosslink that forms in vivo on tail tendon collagen of diabetic rats (Table 2). Additionally, in vitro tests performed at the Picower Institute for Medical Research in New York revealed that some of

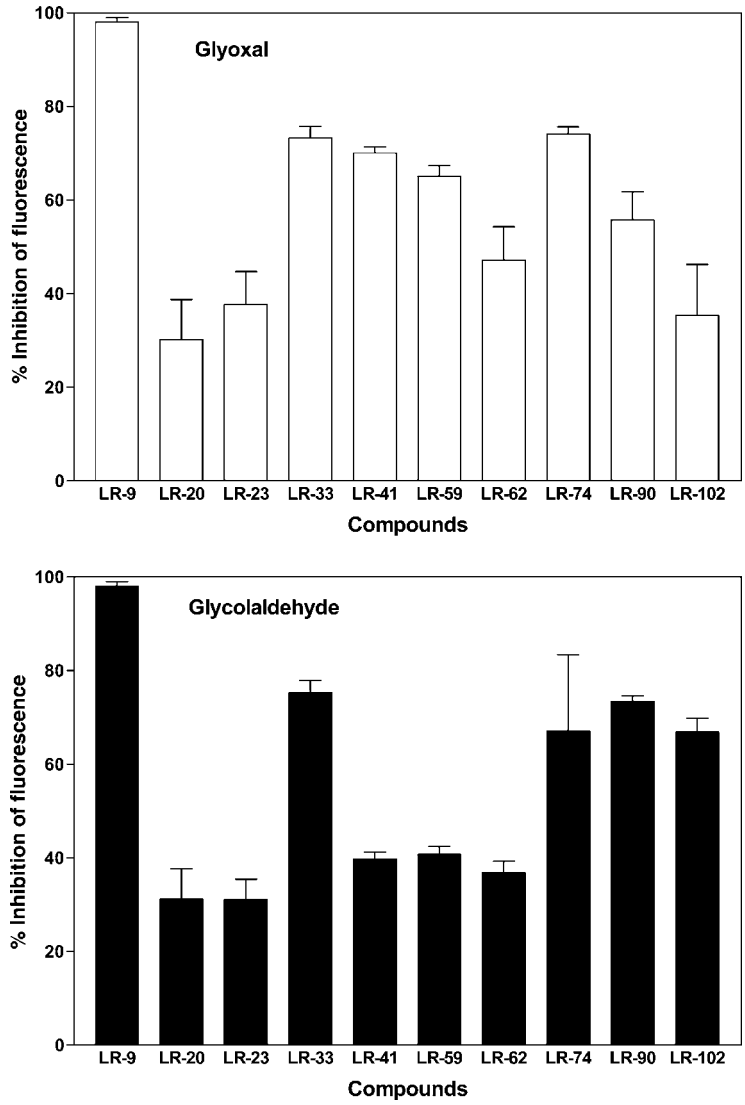


Fig. 9. Inhibition of fluorescent AGE derived from exposure of BSA to GO and GLA. Carbonyl compounds (6.7 mM) and BSA (7.5 mg/ml) were incubated alone or with the inhibitor (6.7 mM) for 7 days at 37 °C in 200 mM sodium phosphate buffer, pH 7.5, containing 0.2/L sodium azide. Fluorescent non-CML modified BSA was measured (triplicate samples) by measuring the inhibition of fluorescence (excitation at 322 nm emission at 408 nm). Top panel demonstrates the percentage of inhibition of control for each compound using glyoxal as the carbonyl compound. Lower panel is from the test performed with glycolaldehyde.

these novel AGE inhibitors, including SMR-5 (5-aminosalicylic acid), can disaggregate fibrillar forms of both native and glycated β-amyloids (S. Rahbar and R. Bucala, unpublished data). Whether these compounds can exhibit the same properties in vivo remains to be investigated in the future (see Table 3)

Conclusion

There is a considerable body of evidence implicating formation and accumulation of advanced glycation end products as a major factor in the development of diabetic complications, atherosclerosis, Alzheimer's disease, and the normal aging process. The significance of this phenomenon becomes more evident where tight association of lipoxidation reactions, over-production of reactive oxygen species (oxidant stress), and over-generation of RCS (carbonyl-stress) with the process of AGE formation are considered. Cell and tissue damage by AGE comes from cross-linking and remodeling of structural proteins such as collagens and metabolic enzymes and affecting their physiological functions. Furthermore, tissue damage particularly in vascular endothelial cells may originate by triggering of key cell signaling systems and stimulation of inappropriate cellular activities through secretion of cytokines and vascular cell adhesion molecules.

Thus, therapeutic interventions should not only target AGE formation and AGE-protein cross-link

Table 3
Effects of novel AGE inhibitor compounds on breaking AGE crosslinks that form in vivo on rat tail collagen

Compound and concentration	Acid solubility test (% increase in solubility)	Papain digestion assay (% decrease in fluorescence)	Pepsin digestion assay (% decrease in fluorescence)
LR-20			
0.1 mM	1.5	18.3	7.1
1.0 mM	7.3	18.4	8.9
10 mM	24	24.3	27.8
LR-23			
0.1 mM	18.3	16.7	7.3
1.0 mM	19.4	25.9	15.5
10 mM	32.7	47.2	18.7
LR-99			
0.1 mM	1.6	23.5	9.8
1.0 mM	7.3	35.9	16.1
10 mM	16	63.6	12.6
LR-102			
0.1 mM	11.3	21.2	12.9
1.0 mM	18.1	22.8	23.8
10 mM	24.2	52.2	37.3
5-ASA			
0.1 mM	0	1	4.7
1.0 mM	5.7	17.3	12.1
10 mM	12.1	17.3	21.1
Metformin			
0.1 mM	0	3.5	3.1
1.0 mM	9.4	16.4	7.5
10 mM	10.3	19.2	12.7

All values were from collagen treated with the compounds relative to untreated tail tendon collagen of diabetic rats.

formation. Considering the complexity of pathways and reactions involved in AGE formation, it is not only the end-products such as AGE and ALE, but also highly reactive carbonyl intermediates responsible for their formation that are toxic to the cells that should be targeted in designing inhibitors that specifically react with each committed step and intermediate products of important pathways. Another factor to be considered is the fact that glycoxidized proteins generate reactive oxygen species (ROS) and induce oxidative stress through the reaction with RAGE. Also, ROS is generated by other reactions in the cascade of AGE formation such as MGO and Schiff's base pathways leading to lipoxidation and oxidative damage to cells. Therefore, strategies such as suppression of receptor signaling pathways (e.g., RAGE antagonists), and the use of antioxidants and α-oxoaldehyde scavengers have been proposed [67,94, 139–142]. The development of inhibitors and AGE-breakers might be difficult as AGE are complex and as there are a variety of crosslinks, thus a variety of mechanism-based-AGE-inhibitors need to be designed and developed. Nonetheless, compounds that possess both metal chelating and carbonyl scavenging properties are ideal to inhibit both oxidative and carbonyl stresses. Such class of compounds may also be effectors of AGE-receptors such as RAGE that is involved in cell signaling pathways [67]. Unfortunately, from a large number of naturally occurring and synthetic compounds reported previously as AGE inhibitors, only the mechanism of action of a few compounds have been studied extensively to date. Unraveling the mechanism(s) of action of these inhibitors is essential for understanding the roles of AGE in the pathogenesis of a number of age-related chronic diseases and to design more effective therapeutic strategies for these diseases in the future.

Booth et al. [112] recently described a new class of post-Amadori AGE inhibitors (termed Amadorins), which block the formation of AGE from Amadori adducts on proteins in the absence of autooxidation of free Schiff-base sugar. Controversial reports on the reaction of PM and MGO have been published recently. Nagaraj et al. [133] found that PM inhibited the formation of MGO-derived AGEs, reduced MG levels in RBCs and plasma from diabetic rats, and isolated a major adduct of reaction between MG and PM (methylglyoxal–pyridoxamine dimer). However, in a most recent study by Khalifah et al. [143,144], little if any post-Amadori inhibition effects by pyridoxamine were detected in their experiments. Also, the authors found no evidence for the formation of covalent adducts of PM with Amadori or related intermediates, but concluded that PM more likely affects glycoxidative AGE formation by interfering with transition metals. In our studies, we have investigated the post-Amadori activities of our novel

aromatic compounds and showed that some of these novel compounds were also potent post-Amadori inhibitors. Compounds like LR-20, LR-74, LR-90, and LR-102 showed greater activity than PM, the prototype post-Amadori inhibitor. The exact mechanism of how these compounds inhibit post-Amadori AGE formation is currently unknown. Thus, in our next series of experiments, we determined if these novel compounds could also function as carbonyl traps of AGE intermediates as well as possessing metal chelation activities.

We have systematically screened our newly developed inhibitor compounds for copper-chelating properties and their ability to react with reactive carbonyl compounds. From 102 compounds analyzed, 11 demonstrated potent copper-chelating properties. In fact, these 11 compounds were among the most active AGE-inhibitor compounds as evaluated by several in vitro assay methods. Additionally, a few of these compounds interacted in vitro with MGO, GO, and GLA. These data confirmed our suspicion that these novel aromatic AGE inhibitor compounds we previously identified by other assay methods indeed possess AGE inhibition properties partly due to inhibition of metal-catalyzed autooxidation reactions. Whether metal chelation alone or other inhibitory mechanisms is responsible for these inhibition properties remains to be investigated in the future. The importance of transition metal chelators as a therapeutic strategy for diseases like diabetes and Alzheimer's disease has already been initiated in vivo with promising results [145,146].

The results of the in vivo investigation indicate that LR-90, one of the more potent novel AGE inhibitor compounds in vitro, markedly inhibited the increase in albuminuria, serum creatinine, serum AGE, and the amount of IgG crosslinked to RBC in diabetic rats without any effect on glycemic control. Additionally, administration of LR-90 prevented the progression of glomerular sclerosis, AGE and collagen deposition in kidney glomeruli, as well as oxidative damage in the renal cortex [129]. Our data suggest that LR-90 reduces in vivo AGE and protein crosslinking without any adverse effects and could be beneficial to the prevention of diabetic nephropathy.

In summary, our studies suggest that most of the novel aromatic AGE inhibitor compounds we previously identified using both fluorescent and immunological methods probably exert their inhibitory actions, either by chelating transition metals ions in the oxidative mechanisms of AGE formation and/or interfering with the reactions of reactive dicarbonyl intermediates of AGE formation, or both. Thus, these novel compounds, particularly those with high chelation activities and carbonyl scavenger properties, should be promising candidate therapeutic agents against various diseases associated with oxidative and carbonyl stress.

Acknowledgments

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