Abstract

Introduction

Aldose reductase (ALR2) is both the key enzyme of the polyol pathway, whose activation under hyperglycemic conditions leads to the development of chronic diabetic complications, and the crucial promoter of inflammatory and cytotoxic conditions, even under a normoglycemic status. Accordingly, it represents an excellent drug target and a huge effort is being done to disclose novel compounds able to inhibit it.

Areas covered

This literature survey summarizes patents and patent applications published over the last five years and filed for natural, semi-synthetic and synthetic ALR2 inhibitors. Compounds described have been discussed and analysed from both chemical and functional angles.

Expert opinion

Several ALR2 inhibitors with a promising pre-clinical ability to address diabetic complications and inflammatory diseases are being developed during the observed timeframe. Natural compounds and plant extracts are the prevalent ones, thus confirming the use of phytopharmaceuticals as an increasingly pursued therapeutic trend also in the ALR2 inhibitors field. Intriguing hints may be taken from synthetic derivatives, the most significant ones being represented by the differential inhibitors ARDIs. Differently from classical ARIs, these compounds should fire up the therapeutic efficacy of the class while minimizing its side effects, thus overcoming the existing limits of this class of inhibitors.

Keywords

Aldose reductase; aldose reductase inhibitors; aldose reductase differential inhibitors; aldose reductase inhibitor patents; phytoparmaceuticals; semi-synthetic inhibitors; synthetic inhibitors.

1. Introduction

Aldose Reductase (alditol:$NADP^+$ oxidoreductase, EC 1.1.1.21, AKR1B1, ALR2) is a small, cytosolic, monomeric enzyme which belongs to the aldo-keto reductase superfamily. It catalyzes the NADPH-dependent reduction of a wide variety of aldehydes to their corresponding alcohols, showing a broad substrate specificity. Since its identification, in the early sixties, this enzyme has been always linked to the development of long term diabetic complications. Actually, ALR2 is the key enzyme of the so called polyol pathway, shown in Figure 1 [1].

It converts glucose to sorbitol, which is then oxidized to fructose by sorbitol dehydrogenase (L-iditol:NAD^+ 5-oxidoreductase, EC 1.1.1.14, SD). As ALR2 shows a low substrate affinity for glucose, the conversion of glucose to sorbitol through this metabolic pathway is generally non-significant under euglycemic conditions. In fact, ALR2 must compete directly with the hexokinase of the glycolytic pathway and, as the substrate affinity of hexokinase is greater than that of ALR2, glucose is preferentially phosphorylated with ATP by this enzyme. On the contrary, under hyperglycemic conditions, hexokinase is rapidly saturated and the polyol pathway becomes operative. Sorbitol is formed more rapidly than it is converted to fructose but its polarity hinders an easy penetration through membranes with a subsequent removal from tissues by diffusion. Therefore, it tends to accumulate within the cell increasing its osmolarity.

In addition to the osmotic imbalance, an increase in the activity of the polyol pathway during hyperglycemia causes a substantial imbalance in the free cytosolic coenzyme ratios $NADPH/NADP^+$ and $NAD^+/NADH$. This alteration in the redox state of pyridine nucleotides induces a state of pseudohypoxia, which contributes to the onset of hyperglycemic oxidative stress through the accumulation of reactive oxygen species (ROS). ROS, in turn, trigger activation of downstream mechanisms, namely protein kinase C (PKC) isoforms, mitogen-activated protein kinases (MAPKs) and poly(ADP-ribose)polymerase (PARP), as well as the inflammatory cascade, which sustains the pathogenesis of diabetic tissue injury and disfunction. Furthermore, the increase in fructose levels connected with the polyol pathway activation accelerates the development of

these complications, since fructose and its metabolites are almost 10 times more potent non-enzymatic glycation agents than glucose [2-8].

The osmotic and the oxidative stress, both generated by the activation of ALR2, strongly participate to the onset of diabetic impairments, affecting mainly the nervous, renal, vascular and ocular systems.

Besides these biochemical evaluations, additional approaches involving genetic analysis have provided unambiguous evidence of the role of ALR2 in the development of diabetic complications, and the correlation between overexpression of the human ALR2 gene and the likelihood of the development of complications among diabetic patients is now a matter of fact. [9-12]

Accordingly, this enzyme stands up as the key and critical checkpoint for the main pathological changes affecting over time diabetic tissues, and its inhibition represents an effective tool to prevent or at least delay the progression and the severity of diabetic complications [13,14]. This is why a huge effort is being done, both by academic and industrial researchers, to disclose novel compounds able to inhibit it.

A throughout literature survey having ALR2 as the main research topic [15] revealed that, starting from the mid sixties, the scientific interest towards this enzyme and its inhibitors grew up quickly, reaching a peak of maximum production around the nineties (Figure 2). During these years, a great amount of structurally different compounds have been disclosed and patented as potent and effective ALR2 inhibitors (ARI), the deepest studied ones being reported in Figure 3.

However, despite the expectancies, a true and widespread ARI therapy never became an established fact and, to date, epalrestat (Figure 3) is the only agent successfully marketed in Japan, India and China for the treatment of diabetic neuropathy [16].

Products that appeared to be promising during in vitro studies or in trials with animal models often failed to proceed any further showing uncertain results in clinical trials with humans. Their failure was often due to the emergence of adverse side effects: the clinical development of sorbinil (**1**, Figure 3), the progenitor of the hydantoin-based inhibitors, was discontinuated because of

hypersensitivity reactions related to the presence of hydantoin ring [17]. Tolrestat (**2**, Figure 3), the key representative of the carboxylic acid inhibitors class, displayed severe liver toxicity [18] , and the same was also true for the parent acid zopolrestat (**5**, Figure 3) [19]. In addition, a significant number of compounds under investigation showed limited clinical efficacy, often caused by an inappropriate dosing schedule of the inhibitor or by an unfitting pharmacokinetic tethered with its absorption, distribution, metabolism, and excretion properties. The observed difficulties led to a general and understandable decrease of scientific interest on this enzyme and its inhibitors, which dragged out till the beginning of 2000's (Figure 2).

Over the last two decades, additional studies on the functional engagement of ALR2 allowed to deepen and comprehend thoroughly its physiological role. The novel evidences revamped the interest of medicinal chemists in the ARI field, surging ahead the search of novel active compounds (Figure 2).

All considered, ALR2 can be now advised as a Janus-faced enzyme, being involved in biochemical pathways leading to both pathological and resolutive conditions.

Actually, besides ruling the polyol pathway, this enzyme represents a key component of the complex antioxidant cell defense system including aldehyde dehydrogenases (ALDHs), which catalyze the oxidation of aldehydes to their corresponding acids [20,21], glutathione-S-transferases (GSTs), which catalyze the conjugation of aldehydes with glutathione [22,23], and other aldo-keto reductases (AKRs), above all aldehyde reductase, ALR1, (EC 1.1.1.2, ALR1), which shows a high degree of structural homology with ALR2, possessing a 65% identity in the aminoacid sequences [24-28]. Accordingly, ALR2 is highly efficient in reducing toxic aldehydes, such as 4-hydroxy-2,3-nonenal (HNE), methyl glyoxale, and 3-deoxyglucosone, arising in large quantities from pathological conditions connected with oxidative stress and responsible for the formation of protein cross-links and advanced glycation end products (AGEs). The enzyme ensures an efficient and complete removal of these end products of lipid peroxidation, thus playing a significant detoxifying function which becomes highly relevant when other antioxidant mechanisms are overwhelmed.

However, at the same time, it catalyses the reduction of glutathione conjugates of unsaturated aldehydes, like GS-HNE, showing in most cases a catalytic efficiency higher than that displayed against the parent free aldehyde. In this case, the corresponding glutathione conjugates of reduced aldehydes, like GS-DHN, turns out to be responsible for cytotoxicity through modulation of the NF-*k*B/PKC/IKK/PLC signalling pathways, and this evidence demonstrates unequivocally the causal consequence between ALR2 activity and inflammatory and cytotoxicity signalling [29-31]. Thus, while under hyperglycemia this enzyme is the main plugger of the development of chronic diabetic complications, it is also a crucial promoter of inflammatory and cytotoxic conditions, even under a normoglycemic status.

Therefore, besides allowing to manage diabetic complications, ALR2 inhibition may be also considered a useful strategy to control pathological conditions emerging from tissue inflammation like atherosclerosis, sepsis, arthritis and also cancer. This evidence offers a novel and even wider chance of use to the class of ALR2 inhibitors, which now have been increasingly releasing from the restricted range of long term diabetic complications treatment in which they have been normally confined.

However, the multifaceted functional role of ALR2 still makes the obtainment of active and safe inhibitors highly demanding, thus legitimating the paucity of effective compounds developed to date. This review overviews literature data on patents and patent applications having ALR2 inhibitors as the core theme, filed during the last five years, with the aim to update readers working on this topic providing new perspectives on the existing facts.

2. Aldose Reductase and its Inhibitors

Compounds able to inhibit ALR2 have been described steadily throughout years by researchers belonging to both academic and industrial institutions. At a glance, they seem to be significantly different from a chemical point of view. Nevertheless, they all must share few key structural

features which turn out to be essential for a profitable interaction with the active site of the enzyme, discussed hereafter.

The X-ray crystallographic analysis of the ALR2 holo-form (PDB entry: 1abn [32]) reveals that ALR2 adopts a Triose phosphate IsoMerase TIM-barrel conformation, folding up in a typical eight-stranded β/α-barrel, to which a small β-sheet capping the N-terminal end, residues 2–14, and a C-terminal extension, residues 275–315, are added. The active site is located at the C-terminal side of the barrel, deeply buried into it. The cofactor binds to ALR2 in an extended conformation, straddling the barrel and projecting its nicotinamide moiety to the middle of the protein [33-35], where it becomes part of the catalytic site contributing to the reduction mechanism.

The catalytic active site of ALR2 is highly hydrophobic (Figure 4). With the only exception of three polar residues, namely Gln49, Cys298 and His110, the cavity is bordered by aromatic residues, such as Trp20, Tyr48, Trp79, Trp111, Phe121, Phe122 and Trp219, and apolar residues, such as Val47, Pro218, Leu300, Leu301. Therefore, ALR2 shows a marked orientation towards lipophilic substrates. Considering its fundamental role in the aldo-sugar metabolism, such a preference would seem to be rather unusual. Actually, D-glucose is a well-recognized physiological substrate for ALR2 [36], but it is not the preferred one. The affinity shown by the enzyme for its reduction appears low, as an apparent K_m value ranging from 50 to 100 mM testifies [37]. However, the Km for acyclic glucose, the true form of the glucose substrate of ALR2, is 5 μM [38]. This confirms that ALR2 metabolizes this sugar only when its concentration increases to pathological levels, such as under diabetic conditions. Extensive studies by different research groups have clearly demonstrated that ALR2 is highly efficient in reducing short- to long-chain aldehydes, whether saturated or unsaturated, aliphatic or aromatic, either of exogenous origin or potentially arising in large quantities from lipoproteins and membrane phospholipids, as a consequence of pathological conditions connected with oxidative stress. ALR2 also catalyzes the reduction of glutathione conjugates of unsaturated aldehydes, and steroid metabolites such as 3,4-dihydroxyphenylglycoaldehyde, isocorticosteroids, isocaproaldehyde, progesterone and 17α-

hydroxyprogesterone. Noradrenaline catabolites have been identified as further possible endogenous ALR2 substrates [39-46].

The broad substrate specificity shown by ALR2 is achieved through a high degree of plasticity of its catalytic binding site. A lot of crystal structures of this enzyme, obtained in the presence of different compounds, mainly inhibitors, concurred to prove the ability of ALR2 to modify the conformation of this site as a result of an induced-fit adaptability to the ligand. A careful comparison of these conformations led scholars to consider the ALR2 binding site as composed by two distinct portions, commonly named the 'anion binding pocket' and the 'specificity pocket', possessing a with different flexibility.

The 'anion binding pocket', which is bordered by the pyridine ring of the cofactor and the surrounding aminoacids (Figure 4), takes part actively in the catalytic machinery ruled by the enzyme, appearing rather stiff. Actually, it represents the fixed portion of the site which anchors the ligand, either a substrate or an inhibitor, through its functional group. Apart from Trp111, which exposes its π-face to the remaining part of the site and shows slight changes in the position of its side chain, residues lining the 'anion binding pocket' and represented by Trp20, Tyr48, Val47, His 110, and Trp79, maintain their positions unaltered across all the known crystal structures of ALR2. On the contrary, the so-called 'specificity pocket' is highly elastic, and shows frequent changes in its conformation. Residues going from Val297 to Leu300, the flanking Trp219, Cys303 and Tyr309, and, to a lesser extent, the distant Thr113 and Phe122 (Figure 4), host the lipophilic portion of the ligand, exhibiting a marked adaptation to it. In particular, Leu300 plays a determining role as gatekeeper: depending on the rearrangement of its side chain, as well as on the resulting position of the neighboring Cys303 and Tyr309, Leu300 makes the 'specificity pocket' accessible, expanding its width [47-49].

To date, a number of conformations of the active binding site have been identified, both different and recurrent. The most frequent one is the so-called 'holo-conformation', shown not only by the holo-enzyme, but also by the enzyme complexed with different ligands such as, for example,

glucose-6-phosphate (PDB entry: 2acq [50]), cacodylate (PDB entry: 2acr [50]), citrate (PDB entry: 2acs [50]), and sorbinil (PDB entry: 1ah0 [51]). In this conformation, the binding site is quite limited as Leu300, turning its side chain towards Trp111 (Figure 4), shortens the extension of the 'specificity pocket'. The ligand, anchored to the 'anion binding pocket', mainly occupies this part of the site, and if its size exceeds that of the pocket, it may protrude above the protein.

When ALR2 binds the inhibitor tolrestat (PDB entry: 1ah3 [51]), a different conformation of the active site is observed. Besides the kinked Leu300 side chain, three additional residues, namely Phe122, Cys303 and Tyr309 show their side chains shifted, thus opening up a wider 'specificity pocket' which can host the lipophilic portion of the inhibitor.

Additional protein conformations have been obtained in the complexing of ALR2 with different inhibitors [52,53] and also in these examples, the main changes involve the C-terminal loop region of the enzyme, including the side chains of Ala299 and Leu300, which widen the 'specificity pocket' of the active site, adopting a completely different conformation.

More recently, an unusual conformer of ALR2 was determined by G. Klebe and coworkers who, by crystallizing the enzyme with a carboxylic acid inhibitor bearing a naphtho[1,2-*d*]isothiazole core, disclosed the opening of a novel sub-pocket which extends the 'anion binding pocket' through the rotation of the indole moiety of Trp20. This achievement is truly surprising and unexpected, as it affects a portion of the binding site usually found to be rigid and unbendable, proving once more the high flexibility of this enzyme [54,55].

From the medicinal chemist's point of view, the marked induced-fit adaptability of ALR2 is attracting and fascinating. Actually, as it is hard to predict the binding mode of the ligands even through docking and virtual screening, the development of novel, effective inhibitors represents an intriguing and stimulating challenge [56,57], which inevitably results in a plethora of chemically different compounds [58-69]. Those developed over the last five years, which became the main core of patents filed, have been analyzed and reviewed hereafter grouped on the basis of their origin: natural derivatives, semi-synthetic compounds obtained through suitable modifications of naturally

occurring scaffolds, and synthetic inhibitors.

3. Aldose Reductase Inhibitors of Natural Origin

In accordance with an increasingly pursued drug development trend, the potential role of phytopharmaceuticals has been re-discovered in these last few years also in the ARI field. Such a tendency fully agrees with the current policy of the World Health Organization (WHO) on the traditional and complementary medicine, whose development is strongly supported [70]. Actually, WHO promotes the rational, methodological and safe use of traditional herbal medicines, whose integration into the health care practices of both industrialized and developing countries is highly recommended. Moreover, as traditional herbal medicines are already frequently consumed in the developing countries, their use could easily become an accepted and affordable therapeutic strategy to increase access to health care even by poor populations.

Over the last five years, a number of either aqueous or organic extracts of different part of plants have been described and patented for their properties to inhibit ALR2. All the patents filed, which represent by far the substantial majority of those having an ARI as the main object, are by different researchers from Western Pacific States like China, Japan and Republic of Korea, that is countries where traditional herbal medicines are widely used. In particular, it is the Korean researchers who take the lion's share.

Most of the active ingredient of the patent filed are represented by mixtures of polyphenolic compounds, and the described compositions are claimed to be administered either as a pharmaceutical formulation or as a dietary supplement. Besides a demonstrated ALR2 inhibitory activity, quantified in the micromolar/submicromolar range by the different authors, polyphenolic compounds possess well known antioxidant properties as they are able to scavenge excess reactive oxygen species (ROS). Diabetic patients might certainly benefit from a therapy supplemented with antioxidants, being ROS one of the underlining causes of tissue damages under hyperglycemic conditions. Moreover, it is worth mentioning that ALR2 undergoes an oxidative induced

posttranslational modification involving a critically active cysteine thiol (CYS298), which regulates both substrate and inhibitor binding activity of the enzyme. The resulting oxidized form of ALR2 shows an increase in Km for aldehyde substrates and a marked reduction in sensitivity to ARIs, thus compromising the therapeutic effectiveness of these compounds [71,72]. Therefore, the development of more effective therapies to prevent long-term diabetic complications should profitably combine ARIs and antioxidants, in order to keep the enzyme in the reduced form.

Kim Jin Suk and co-workers, from Korea, focused on *Hedera rhombea*, an evergreen plant native of the coast of East Asia and belonging to the Araliaceae family, whose extracts are claimed by the authors as effective ALR2 inhibitors able to prevent or treat diabetic complications [73]. Sun Seong Lim and co-workers, from the Hallym University, took into account the Valerianaceae *Nardostachys chinensis*, commonly used as an analgesic herb in the Ayurvedic tradition [74]. The use of a phytocomplex obtained from the plant and containing protocatechuic acid **8**, caffeic acid **9**, chlorogenic acid **10** and its methyl ester **11**, debilone **12**, nardoxide **13**, and 1,5-di-O-caffeoyl-quinic acid **14** (Figure 5), is recommended by the authors to alleviate, prevent or treat diabetic complications. Moreover, it can be also exploited as an active ingredient to enrich foodstuff, thus obtaining a novel type of functional food able to provide benefits to diabetic people. The same authors analysed also the perennial *Colocasia esculenta*, an herbaceous plant belonging to the Araceae family and native to South-East or southern Central Asia, which is commonly used as a staple food in tropical and sub-tropical regions throughout the world [75]. Extract obtained from *Colocasia esculenta* has a complex chemical profile comprising one aminoacid, L-tryptophan **15**, and a number of polyphenolic derivatives like vitexin **16** and its 6-isomer isovitexin **17**, the corresponding 2-cathecol-analogues orientin **18** and isoorientin **19**, the luteoline derivatives **20** and **21**, and the cynnamic derivatives **22-24** (Figure 6). Similarly to other natural phytocomplexes, the one obtained from *Colocasia esculenta* is able to inhibit ALR2 activity, thus being suitable for preventing and treating diabetic complications. The same functional profile has been also claimed by the same authors for extracts obtained from *Syringa oblata* [76]. This is a deciduous shrub of the

Oleaceae family, native to China and commonly called lilac, which provides an extract endowed with anti-oxidant activity. The concomitant ability to block the catalytic activity of ALR2 and the formation of advanced glycation end products makes this extract a privileged formulation to be administered to diabetic people.

Pheophorbide A **25**, represented in Figure 7, has been claimed by Gwang Won Lee and co-workers as both an aldose reductase and an α-glucosidase inhibitor [77]. Accordingly, it is useful to treat or prevent metabolic disorders due to hyperglycemic condition. The compound may be obtained from the filamentous green algae *Capsosiphon fulvescens*, belonging to the Ulvophyceae class and native to the North Atlantic and the Northern Pacific, including Korea and Japan. Subsequent steps of extraction, accomplished by 70% ethanol, water and chloroform, followed in turn by ion exchange fractionaction and gel filtration purification allow to get to the target compound in its pure and exploitable form.

Additional compounds from natural sources have been patented as ALR2 inhibitors by Sang Hyeon Lee and So Yeon Mok, from the Chung-Ang University [78]. Flowers obtained from *Rhododendron mucronulatum* were firstly extracted with methanol, then fractionated with ethyl acetate, to obtain an active fraction exhibiting ALR2 inhibitory activity in the submicromolar range (IC_{50} 0.15 μg/mL). A closer inspection of the active fraction revealed that it was mainly composed by the 3-O-rhamnosyl derivatives quercitrin **26** and myricitrin **27**, accompanied by the corresponding aglycones quercetin **28** and myricetin **29**. The benzopyran-3-one derivative kaempferol **30** and its 3-O-rhamnosyl derivative afzelin **31** were present as well (Figure 8). Tested as separate compounds against the target enzyme, they all turned out to be active. The best inhibitory activity was displayed by the glycosylated derivative quercitrin (IC_{50}: 0.13 μg/mL), followed by afzelin (IC_{50}: 0.31 μg/mL), and quercetin (IC_{50}: 0.48 μg/mL). Kaempferol retained a sub-micromolar efficacy (IC_{50}: 0.79 μg/mL), while the lowest efficacy was observed for myricitrin (IC_{50}: 2.67 μg/mL) and its aglycone myricetin (IC_{50}: 11.92 μg/mL). Taken as a whole, the phytocomplex is suggested by the authors as an active ingredient for preventing and treating diabetic complications.

In 2013, the Chinese researchers Xiaoqing Zou and co-workers proposed to exploit a crude extract of the bush *Rubus suavissimus*, belonging to the Rosaceae family and known as Chinese Blackberry [79]. The *Rubus suavissimus* leaves are commonly used as a traditional Chinese herbal tea, characterized by a unique sweet taste thanks to the presence of the high sweetness rubusoside, a diterpene glycoside. According to the authors, the gathering of tea glycosides, a mixture of different polyphenols, L-theanine **32**, and caffeine **33** (Figure 9), as they result from the crude extract, represents an active formulation to use for the treatment of diabetic people thanks to its ability to inhibit the key enzyme ALR2.

More recently, Liu Hongwei and co-workers analyzed a number of terpenoid compounds, extracted and characterized from the fungus *Ganoderma lucidum*, the woody mushroom known as 'Lingzhi' in Chinese, 'Reishi' in Japanese, and 'Yeongji' in Korean. These include the ganomycines B, I, and J **34-36** (Figure 10) [80], several derivatives bearing the lanost-8-en-26-oic acid core, **37-47**, including the lucidenic and the ganoderic acid, **46** and **47**, respectively (Figure 11), as well as products derived from the reciprocal combination of these derivatives **48-51** (Figure 12) [81]. They are all able to inhibit the target enzyme, thus are claimed by the authors as possessing a wide application prospect for the treatment of diabetic complications.

Zang Kun and co-workers, in 2016, focused on the polyphenolic ALR2 inhibitor tiliroside **52**, kaempferol-3-O-β-D-(6'-O-trans-*p*-hydroxylcinnamyl)-glucopyranoside (Figure 13) [82]. As this compound can be easily obtained from the flowers of the small evergreen bush *Edgeworthia gardneri*, the authors suggest to use these parts of the plant to have a functional tea, whose intake may help to control the development of long term diabetic complications.

Murai Hiromichi and co-workers, from Japan, patented a 30% ethanol extract of *Prunus lannesiana* flowers [83]. Actually, it proved to inhibit ALR2, exhibiting an in vitro IC_{50} value of 6.25 mg/mL, thus it has been taken into account for the obtainment of a novel antidiabetic formulation.

Julius Angeline from India tested different polyphenol derivatives against ALR2, identifying a small number of compounds showing inhibitory efficacy in the nanomolar range [84]. These

include the cyclopenta[*c*]pyran-5-oles agnuside **53** (IC_{50} 22.4 nM) and picroside II **54** (IC_{50} 130 nM), and the croman-3-ones derivatives eupalitin-3-O-Galactoside **55** (IC_{50} 27.3 nM) and 7-O-methylwogonin **56** (IC_{50} 108 nM) (Figure 14). By combining agnuside and eupalitin-3-O-Galactoside the author succeeded in obtaining an active phytocomplex which proved to inhibit ALR2 from both ARPE19 human retinal pigment epithelial cell line and diabetic lens, showing IC_{50} values lower than the commercially available ALR2 inhibitor Epalrestat.

4. Aldose Reductase Inhibitors Derived from Natural Compounds

In 2013, Milan Stefek and co-worker exploited the natural scaffold of quercetin to develop a novel class of ALR2 inhibitors [85]. By functionalizing at least one phenolic residue of the flavonolol derivative with electrophilic residues like the 2-chloro-1,4-naphthoquinone, the 4-O-acetylferuloyl chloride and the 3-chloro-2,2-dimethylpropanoyl chloride, the author succeeded in obtaining potent inhibitors endowed with a concomitant antioxidant efficacy. Representative example of the class, **57-59**, characterized by the presence of the mentioned residues in position 4' of the nucleus, are depicted in Figure 15. As stated previously, adding antioxidant properties to compounds able to block the polyol pathway *via* ALR2 inhibition can ameliorate the pharmacological profile of the resulting derivatives, since in this metabolic pathway an increased quantity of ROS can be produced, worsening diabetes-induced tissue damage at different levels. Furthermore, in principle, treatment with antioxidants can keep the enzyme in the reduced form, thus preventing the development of drug resistance following its oxidative modification at the CYS298 residue.

Additional compounds obtained by chemical modification of natural scaffolds have been patented by the Chinese researchers Heru Chen and co-workers [86-88]. In this case, the starting natural lead is represented by α-cyano-4-hydroxycinnamic acid. Thanks to a three steps synthetic procedure, achieved by using firstly N-Boc-diamine compounds and lastly both natural and unnatural N-acyl-α-amino acid derivatives, the lead gives rise to a novel series of inhibitors having compound **60** as

the representative example (Figure 15). This latter proved to inhibit ALR2 potently, showing an IC_{50} value of 72.7 nM.

To develop a novel class of ALR2 inhibitors, Daniel Labarbera and Mark Petrash took inspiration from β-glucogallin **61**, 1-O-galloyl-β-D-glucose, naturally occurring in gooseberries, *Emblica officinalis* [89,90] and previously described by the same authors as an effective ALR2 inhibitor [91]. Moving from the natural hit, they obtained a novel series of polyphenolic compounds, **62**-**67** (Figure 16), by tying together the two key portion of **61**, namely the sugar moiety and the phenolic ring, with different heteroaromatic linkages. Among the synthesized compounds, the β-glucogallin amide **61** proved to be active against the target enzyme, showing no inhibitory efficacy against the related aldo-keto reductases AKR1B10 and AKR1A1. Moreover, it demonstrated to block sorbitol accumulation both in Raw264.7 murine macrophages and in lens obtained from PAR40 transgenic mice, expressing human ALR2, once excised and cultured ex vivo. These experimental results provide the evidence that the analogues of β-glucogallin proposed by the authors might be profitably exploited for the prevention, the treatment and the prophylaxys of chronic diabetic complications, in particular those affecting the visual system.

4. Aldose Reductase Inhibitors of Synthetic Origin

Carboxylic Acid Based-Inhibitors

During the last five years a number of synthetic compounds have been designed and patented as ALR2 inhibitors, mainly belonging to the carboxylic-type class.

Inspired by the well-known inhibitor zopolrestat (Figure 3), Banavara L. Mylari and co-workers designed a novel class of carboxylic acid inhibitors with the general formula **68** (Figure 17) [92-93]. In this series, the phthalazine heterocyclic core of the literature compound was replaced with the bioisosteric pyrazino[2,3-*c*]pyridazine ring. Thus, the compounds should theoretically share the binding mode of zopolrestat to the ALR2 active site, in which the carboxylic function anchors the inhibitor to the anionic binding site and the benzothiazole ring is projected into the ‘specificity

pocket' lined by Leu300 and Trp111 (Figure 4). Compound **69**, representative of the whole series, proved to block nearly completely (93.6±2.8%) the catalytic activity of the target ALR2 when tested at 1 μM concentration. Thanks to their functional profile, the compounds are proposed as novel drug candidates for the treatment of pathological conditions promoted by an aberrant ALR2 activity, including cardiovascular and renal disorders, tissue damage, cancer and, obviously, long term complications arising from diabetes. Mylari suggests also to use both the representative 2-(8-oxo-7-((5-trifluoromethyl)-1*H*-benzodimidazol-2-yl)methyl)7,8-dihydropyrazin[2,3-*c*]pyridazine-5-yl)acetic and the parent zopolrestat as water soluble salts [94,95]. Actually, by using suitable aminoacids like arginine, lysine, metformin, aspartic and glutamic acid, but also amino derivatives as glucosamine and glucamine, these compounds can be converted into highly soluble derivatives. Once administered orally, they can be promptly absorbed increasing significantly the bioavailability of the parent compounds. In addition, they turns out to be especially suitable for parenteral administration.

Shoshana Shendelman recently patented two novel series of phthalazino and pyrazinopyridazino derivatives [96]. Although closely recalling compounds described by Mylary and co-workers, in their heterocyclic portion, the novel derivatives possess a boronic residue that replace the carboxylic acid moiety. This gives the ARI field a rather new chemical approach, offering the opportunity to pioneer the use of this fragment for the obtainment of active inhibitors. Actually, although still underutilized in therapeutics, the boron atom is characterized by an empty p-orbital, which can be easily occupied by a lone pair from nucleophiles like the aminoacid residues surrounding the catalytic ALR2 pocket. This allows to hook firmly the inhibitor to the target, thus resulting in principle in highly effective compounds. Besides the free boronic acids, **70**-**72**, the author propose the use of the corresponding 1,3,2-dioxaborolanes, **73**-**75** (Figure 17). Lacking the ionizable group, reasonably uncharged at physiological pH values, these compounds should exhibit a more favorable pharmacokinetic profile.

Further structural modification of the progenitor zopolrestat were made by Andrew Wasmuth and co-worker, who replaced the phthalazine central core with a thieno[3,4-*d*]pyridazine nucleus, thus getting to a novel class of effective inhibitors almost equipotent to the known lead [97]. Different 5:6 heterocyclic substituents were inserted in position 3 of the main core including the benzothiazole ring, as in the known lead (compound **76**, Figure 17) but also the benzofurane (compound **77**, Figure 17) and the benzothiophene (compound **78**, Figure 17) residues, which were functionalised in turn, mainly with halogen atoms.

The most innovative examples of compounds patented among the carboxylic acid inhibitors are represented by the pyrazolo[1,5-*a*]pyrimidine derivatives of general formula **79** (Figure 17), described by Umberto Mura and co-workers in 2014 [98]. Thanks to this patent, and first in the ALR2 inhibitors field, the authors launched the concept of Aldose Reductase Differential Inhibitors (ARDIs) as a novel class of compounds able to selectively inhibit the catalytic activity of the enzyme depending on the specific substrates is going to be transformed. This approach, which may be in principle adopted for any enzyme able to act on different substrates, appears especially tailored for ALR2, being an aspecific enzyme. Differently from ARIs developed till now, characterized by high but unspecific binding affinity against the target enzyme, ARDIs should show a differential intra-site binding affinity, being able to inhibit the catalytic activity of aldose reductase against glucose and glutathione conjugates while leaving unaltered, or, in any case less affected, the reductive activity of the enzyme towards toxic hydrophobic aldehydes. Accordingly, using an ARDI, it is possible in principle to prevent both hyperglycemia-induced cell injury and inflammatory and cytotoxic signalling, without affecting cell antioxidant defense. Among the synthesized compounds, derivative **80**, 4-(4-chlorobenzyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-6-carboxylic acid, emerged as a new and viable lead, proving to exert its inhibition toward the catalytic activity of aldose reductase against both L-idose and GS-HNE but not HNE.

Non-Carboxylic Acid-Based Inhibitors

A great prominence has been given in the past to the 2,4-thiazolidinedione ring system, considered as a useful template for the design of antidiabetic agents used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). These compounds act on peroxisome proliferator-activated receptor γ (PPARγ), improving glucose utilization without stimulating insulin release. Furthermore, as this heterocyclic core may be considered to be a hydantoin bioisoster, it became also a privileged scaffold for the synthesis of novel ARIs, potentially devoid of unwanted hypersensitivity side reactions typical of hydantoin-type inhibitors.

This is why, during the last decade, several thiazolidinediones have been recognized as effective ALR2 agents. In 2014, Puroshottam and co-workers patented a novel class of 2,4-thiazolidinediones [99]. The authors claimed the compounds as multi-effective, being able to inhibit ALR2 and, at the same time, exert an agonistic efficacy toward both the alpha and the gamma PPAR receptor subtype. The lead compound **80**, (E)-4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenyl 2-chlorobenzoate (Figure 18), exhibited an IC_{50} value of 1.82 μM when tested against ALR2, and a maximal efficacy of 84.5% and 89.5% when evaluated against PPAR-α and PPAR-γ, respectively.

An additional patent filed in the last five years describes a novel class of 5-phenylpyrrole derivatives of general formula **81** (Figure 18), claimed as effective inhibitors by the Chinese researchers Zhiming Xiu and co-workers [100], although seemingly devoid of any ionizable residue, which is generally acknowledge as a key structural moiety required to interact with the catalytic binding site of the enzyme.

A number of gel preparations for the topical administration of ALR2 inhibitors to the eye have been described, developed especially for the targeted therapy of diabetic complications affecting the visual system [101-104]. In particular, Wyman and Bellavia propose to use the 2-methylsorbinil, 2R,4S-6-fluoro-2-methyl-spiro[chroman-4,4'-imidazolidine]-2',5'-dione **78** (Figure 18), as the reference compound [103,104].

Satish Srivastava and Kota Ramana proposed to exploit well known previously developed ARIs, including sorbinil, ponalrestat, epalrestat, tolrestat, zopolrestat, zenarestat, minalrestat, fidarestat

(Figure 3), for the treatment of cancer, and in particular the one affecting colon, founding their claim on the key role played by ALR2 in cytotoxic signaling [105]. Tested against human colon cancer Caco-2 cell lines, ALR2 inhibitors like sorbinil and tolrestat proved to prevent PGE2 production, Cox-2 activity, growth factor-induced activation of both Nf-kB and PKC, as well as accumulation of cells in S-phase, G2/M phase and G1 phase. These achievements clearly grant prominence to the relevant role played by ALR2 in mediating cellular cytotoxicity signalling, thus demonstrating unambiguously that ALR2 inhibitors can be successfully used not only to prevent or delay long term diabetic complications but also to counteract inflammation and cytotoxicity arising from glutathione conjugates of reduced aldehydes, produced by ALR2 itself.

5. Expert Opinion

The patent literature survey here reported gives a temporal view of the progress achieved in the development of the ALR2 inhibitors field. The number of patent filed over the last five years demonstrates that, after decades of uncertainty, scientific interest for ALR2 and its inhibitors has resurged and almost brought back to the golden era of the nineties. Patents and patent applications emphasizing natural products and plant extracts are the prevalent ones, coming from a diverse pool of global applicants with regions like Korea and China staking strong claims in the space. The use of phytopharmaceuticals represents therefore an increasingly pursued therapeutic trend also in the ALR2 inhibitors field, plainly in line with the WHO current policy which promotes the rational, methodological and safe use of traditional herbal medicines, highly recommending their integration into the health care practices of both industrialized and developing countries. Besides a therapeutic function, the use of natural compounds and plant extracts may have also a significant prophylactic role. Actually, thanks to their dual efficacy, as antioxidant and ALR2 inhibitors, these compounds may protect patients from the development of chronic complications by shutting down oxidative stress and inflammatory changes. In particular, their prophylactic use could be of compelling

relevance for people whose polymorphisms analysis of the aldose reductase regulatory gene emphasises the likelihood of the development of long term complications.

In terms of synthetic compounds, the most innovative ones are doubtless represented by the differential inhibitors ARDIs, proposed by Mura and co-workers in 2014. Differently from classical ARIs, characterized by high but unspecific binding affinity against the target enzyme, these compounds aim to show a differential intra-site binding affinity, being able to inhibit the catalytic activity of aldose reductase against glucose and glutathione conjugates while leaving unaltered the reductive activity of the enzyme towards toxic hydrophobic aldehydes. In doing so, ARDIs should fire up the therapeutic efficacy of the inhibitors while minimizing their side effects, thus overcoming the existing limits of this class of therapeutics. Certainly, getting to novel and effective ARDIs is a highly challenging issue. Due to the marked induced-fit adaptability of the enzyme binding site, identification of compounds able to show a differential intra-site binding affinity paves the way for intensive research efforts, being neither easy nor fast.

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