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In vitro evaluation of 5-arylidene-2-thioxo-4-thiazolidinones active as aldose reductase inhibitors

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ABSTRACT

2-Thioxo-4-thiazolidinone derivatives were evaluated as aldose reductase inhibitors (ARIs) and most of them exhibited good or excellent in vitro efficacy. Out of the tested compounds, most N-unsubstituted analogues were found to possess inhibitory effects at low micromolar doses and two of them exhibited higher potency than sorbinil, used as a reference drug. The insertion of an acetic chain on N-3 of the thiazolidinone scaffold led to analogues with submicromolar affinity for ALR2 and IC_{50} values very similar to that of epalrestat, the only ARI currently used in therapy.

Aldose reductase (EC 1.1.1.21, ALR2) is an aldo-keto reductase which catalyses the NADPH-dependent reduction of glucose to sorbitol in the first step of the polyol pathway. Sorbitol is subsequently oxidized to fructose by sorbitol dehydrogenase with concomitant reduction of NAD^+. Under conditions of hyperglycaemia, such as in diabetes mellitus (DM), increased flux of glucose through this metabolic process occurs in tissues possessing insulin-independent glucose transport (retina, lens, kidney, peripheral nerves) and this has been shown to be critically linked to the aetiology of hyperglycaemia-induced long-term diabetes complications.[1–3] In fact, the intracellular accumulation of sorbitol, which is not diffusible through biomembranes, leads to osmotic changes and cellular damage, particularly in lenses. Moreover, the concurrent NADPH deprivation causes alterations in cellular redox potentials and in the activity of other NADPH-dependent enzymes, such as nitric oxide synthase and glutathione reductase, thus inducing intracellular oxidative stress and producing changes in cytokine signalling. The increased production of fructose can also cause pathological changes, by promoting protein glycation and formation of advanced glycation-end products (AGEs) and thus causing alterations in protein functions. These effects result in oxidative stress, inflammation and vascular damage which trigger a sequence of tissue dysfunctions and thus are responsible for the development of atherosclerosis, retinopathy, cataracts, nephropathy, neuropathy and for increased risk of myocardial infarction and stroke.[1–6]

On these bases, ALR2 is considered an attractive molecular target to develop drugs able to prevent the onset and progression of secondary pathologies associated with DM, even in the presence of imperfect control of glycaemia. In fact, although an opportune antihyperglycaemic treatment can delay the emergence of diabetes complications and decreases the risk of microvascular damage, the normalization of glycaemic levels is not always feasible in diabetic patients and the onset of these pathologies, especially macrovascular diseases, is almost unavoidable.[7] In addition, it has been suggested that the hyperglycaemia-induced cascade of phenomena, such as increased production of AGEs and reactive oxygen species, activation of protein kinase C and nuclear factor κB, can affect gene expression leading to molecular changes which could be responsible for the onset of chronic complications even during the antihyperglycaemic therapy.[8] Thus, taking into account the worldwide soaring rates of DM[9] and the seriousness of its complications, it is urgent to find drugs which can control the development of hyperglycaemia-induced damage and the consequent pathologies.

In addition, recently it has emerged that ALR2 may also be upregulated under normal glycaemic conditions and it can be implicated in the development of other pathological processes, such as a number of human cancers, cardiovascular and inflammatory diseases. Thus, ALR2 inhibition may represent a novel approach for the management of these pathologies, although further investiga-

Abbreviations: AGEs, advanced glycation-end products; ALR2, aldose reductase; ARIs, aldose reductase inhibitors; 2,4-TZDs, 2,4-thiazolidinediones.

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tions are ongoing to elucidate the pathophysiological role of ALR2 under normoglycaemic conditions.[10–12]

Over the last three decades, numerous ALR2 inhibitors (ARIs) have been identified, many of which belonging to either carboxylic acid (such as epalrestat; Fig. 1) or cyclic imide (especially hydantoin, such as sorbinil and fidarestat; Fig. 1) classes of compounds.[11,13–15] However, many of the clinically tested ARIs proved to be therapeutically inadequate because of adverse pharmacokinetics, toxic side-effects or low efficacy. The search for new ARIs has been supported by the knowledge of several crystal structures of ALR2/inhibitor complexes. This contributed to the definition of the structural requirements for ALR2 inhibition, which are: (a) a polar 'head' containing an acidic hydrogen which can bind the polar recognition region of the ALR2 active site formed by the positively charged nicotinamide ring of the oxidized cofactor $NADP^+$ and the hydrophilic amino acid residues Tyr48 and His110; (b) a hydrophobic portion, generally containing an aromatic moiety, which can establish hydrophobic interactions with the lipophilic specificity pocket of the enzyme lined with Trp20, Trp111, Phe122, Pro218, Trp219, Leu300.[16–19]

In this context, 2,4-thiazolidinediones (2,4-TZDs) and 2-thioxo-4-thiazolidinones are considered compounds of significant interest as ARIs, since they can act as hydantoin bioisosteres that are potentially devoid of the hypersensitivity reactions imputed to the hydantoin ring, which caused the withdrawal of sorbinil from clinical trials. Following the discovery of glitazones as oral antidiabetic drugs, numerous 2,4-TZDs have been reported or patented as ARIs, many of which are also endowed with antihyperglycaemic effect.[13,14,20] Many effective ARIs also feature among the 2-thioxo-4-thiazolidinone analogues, including epalrestat (Fig. 1) which is the only ARI currently used in therapy.[21–24]

2,4-TZDs and 2-thioxo-4-thiazolidinones share structural features which appear to be optimal for the interaction with the target enzyme. The portion that can interact with the positively charged recognition region of the ALR2 active site consists of the imidic/thioimidic function or an acetic chain inserted on N-3 of the thiazolidinone framework, whereas a lipophilic portion can be easily inserted in position 5 of the pentatomic scaffold in an orientation which proved to be useful to fit the specificity pocket of the target enzyme.[25–28]

We have recently reported numerous 5-arylidene-2,4-thiazolidinediones, which were shown to be efficacious in vitro ARIs at micromolar or submicromolar doses.[26–30] Among them, acids **1** (Fig. 1) were shown to be the most active in vitro ALR2 inhibitors, with submicromolar IC_{50} values. Although the removal of the acetic chain on N-3 of the thiazolidinedione ring led to a generally significant decrease in potency, certain N-unsubstituted derivatives **2** (Fig. 1) were also found to possess appreciable inhibitory effects.[26,29]

In the context of our continuing search for new efficacious ARIs, we decided to evaluate the in vitro ALR2 inhibitory activity of analogous 2-thioxo-4-thiazolidinones **3** and **4** (Fig. 2). This appeared to be a logical continuation of our studies on thiazolidinone derivatives as ARIs, in order both to identify further active analogues and to refine the structure/activity relationships of this class of inhibitors. Most derivatives **3** and **4** possess a 5-arylidene moiety comprising two aromatic rings, since this structural feature proved to favour the activity of the corresponding series of 2,4-TZDs and to be related to high inhibition levels.[26,29] Hydroxy- and methoxybenzylidene substituted analogues have also been included for comparison.

Although most compounds **3** and **4** (with the exception of **3b**) are already known and commercially available,[31] to the best of our knowledge their ALR2 inhibitory activity was not reported up to now. It is worth noting that, out of compounds **4a**, **4b**, **4f–j**, which were studied as antihyperglycaemic agents, **4b**, **4g–j** produced a moderate reduction of glycaemic levels in obese diabetic mice.[32] In the same patent, their inhibitory activity towards cathepsin D was reported. Compounds **4a** and **4b** resulted to be good inhibitors of this enzyme and, consequently, of the formation of β-amyloid protein, which would be expected to be useful in treating Alzheimer's disease.[32] 2-Thioxo-4-thiazolidinones **4a, 4c, 4d**, **4f, 4g** and **4j** were also proposed as zinc-binding ligands which can reversibly bind to a $HisB^{10}$ Zn^{2+} site of the insulin hexamer, resulting in improved stability of insulin preparations.[33] Analogue **4j** resulted to be a good inhibitor of human arylamine N-acetyltransferase 1, which is under study as a new diagnostic marker and drug target in breast cancer.[34]

Rhodanine derivatives **3** and **4** were synthesised for this study by means of the Knoevenagel condensation of the suitable aldehydes with (4-oxo-2-thioxothiazolidin-3-yl)acetic acid or 2-thioxo-4-thiazolidinone, respectively, in refluxing acetic acid in the presence of sodium acetate (Scheme 1).

The results relative to the evaluation of the in vitro ALR2 inhibitory activity of 2-thioxo-4-thiazolidinones **3a–e** and **4a–j** are reported in Table 1. The assay was performed by using highly purified ALR2 from bovine lenses.[35] Sorbinil and epalrestat were used as reference drugs. In Table 1 the IC_{50} values of the previously assayed corresponding 2,4-TZDs (**1**, **2**)[26,29] were also included for comparison and clarity of discussion.

Most of the tested 2-thioxo-4-thiazolidinones **3**, **4** were found to be more effective ALR2 inhibitors than the corresponding 2,4-TZDs (Table 1), analogous to other rhodanine derivatives present

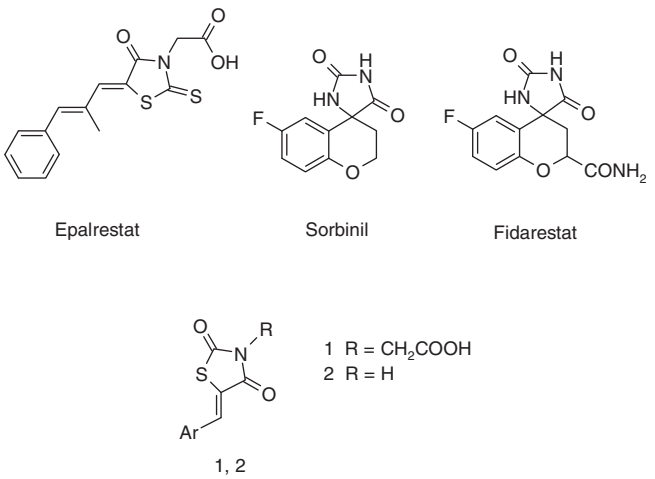

Figure 1. Structures of some ARIs.

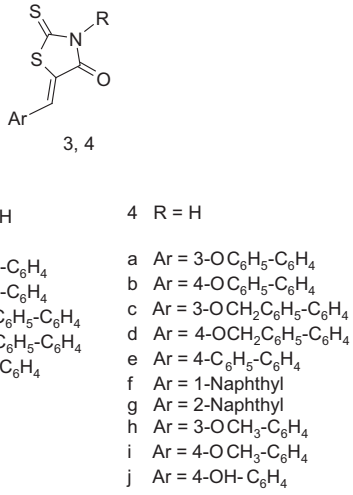

Figure 2. Structures of tested 2-thioxo-4-thiazolidinones **3** and **4**.