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Drug synthesis, separation, and identification of a di-stereoisomeric mixture of spiro-oxalidinonic derivatives of sorbinil by high-performance liquid chromatography

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Abstract
A straightforward and sensitive method for high-performance liquid chromatography (HPLC) was created in order to separate and identify the di-stereoisomeric mixture of a spiro-oxalidinonic derivative of sorbinil. HPLC was carried out using a C18 reversed-phase column. The mobile phase used in the isocratic elution process was a 70:30 v/v combination of methanol and acetonitrile flowed at a rate of 2 mL/min. The procedure delivered the best result among the various attempts made. Two peaks were identified on the chromatogram, attributable to the two di-stereoisomers of the mixture analysed. Analogues of sorbinil, a powerful inhibitor of aldose reductase, have been synthesized and further tested on aldose reductase as mixtures of di-stereoisomers, exhibiting an IC_{50} in the order of the micromolar. The structure of the compounds was checked with nuclear magnetic resonance spectroscopy. The chemical shifts were expressed in ppm scale δ.

KEYWORDS
di-stereoisomeric mixture, HPLC, sorbinil; synthesis, drug

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1. INTRODUCTION

Diabetes mellitus is a heterogeneous syndrome characterized by inadequate insulin synthesis or/and insulin resistance, in which there is an increase of the glucose concentration in the blood and chronic alterations in the carbohydrate, fat, and protein metabolism. As a result, long-term health effects such as the development of retinopathy, nephropathy, neuropathy, and circulatory system disorders can be observed [1]. Therapy

mainly includes the administration of insulin, but this type of treatment fails to prevent diabetes' complications, where high quantities of cellular glucose can be metabolized by aldose reductase (ALR2) to sorbitol [2,3]. The regulation of ALR2 is attributed to reactive oxygen species and nitric oxide (NO). The high sensitivity of the enzyme to these two species is caused by the existence of a residue of high-reactive cysteine (Cys) in its structure [4]. Oxidants like H_2O_2 can inactivate the enzyme as well as cause the glutathiolation of Cys-298. However, by changing the reaction conditions and the NO donor, ALR2 can be *S*-thiolated and, therefore, inactivated or *S*-nitrosated and activated [5]. Based on these observations, it has been believed that NO plays a regulatory role in the intracellular activity of ALR2 and, consequently, in glucose fluxing *via* the polyol pathway [6]. Given that in a hyperglycaemic state, NO release is markedly reduced compared to normoglycemia conditions, it has been proposed that the ALR2 function may be upregulated in the tissues of diabetic people.

Sorbitol cannot easily cross the plasma membrane and is gradually metabolized to fruit sugar (fructose); consequently, it tends to form high concentrations. Sorbitol causes a series of events that lead to irreversible morphological and functional changes. Therefore ALR2 inhibitors (ARIs) could be useful in blocking this metabolic pathway and preventing tissue damage. Since ARIs exert no effect on the plasma levels of glucose (as they do not cause hypoglycaemia), these drugs could be considered co-adjuvants in diabetes therapy [7]. Known ARIs are subdivided into two main types: (i) derivatives of carboxylic acid (zopolrestat, tolrestat, and epalrestat), and (ii) cyclic imides (sorbinil) (Figure 1A). Despite their structural differences, both classes of compounds possess common features: an aromatic planar portion and an acid function able to interact with the enzyme's active site.

Sorbinil is a well-known ARI; it is a spirohydantoin with limited therapeutic value because of its adverse effects, which are caused by the idantoinic group. Numerous analogous, with the idantoinic function substituted with bioisosteres such as thiazolidinedione, oxazolidinone, and succinimide group, have been developed [8]. In these molecules, the chromanonic moiety of sorbinil is substituted in position 2 with different aromatic groups. The same chromanonic moiety was also substituted in position 4 with a 5- or 6-terms spiro-like group linked to an acid function; typical of some ARIs [9]. Some of these compounds had shown a micromolar IC_{50}, but were tested as distereoisomeric mixtures. In an attempt to elucidate how this substitution in positions 2 and 4 of the chromanonic system influences the inhibitory activity of these compounds, it was necessary to determine an analytical method allowing the separation and, therefore, the evaluation of the components of the di-stereoisomeric mixture. We have, herein, carried out the preparation of two spiro-oxalidinonic derivatives, which have shown an IC_{50} of 1.60 and 6.30 μM, respectively, in biological tests.

2. MATERIALS AND METHODS

A solution of commercial 5'-bromo-2'-hydroxyacetophenone (7.90 g; 36.72 mmol) in CH_2CN (10 mL) was agitated at reflux for three days with the appropriate 4-substituted acetophenone (36.72 mmol) and pyrrolidine (0.78g; 11.61 mmol). While spending time to evaporate the solvent, AcOEt and NaOH/HCl removed the deposit. AcOEt was dried, filtered, and evaporated for unrefined oil. Oil was purified by column chromatography with a 95:5 hexane/AcOEt eluent combination. In the sequence of trimethylsilyl cyanide (290 mg; 2.92 mmol) and ZnI_2 (86.7 mg; 0.27 mmol), trimethylsilyl cyanide was added to a solution of appropriate chromanone (1.95 mmol) at ambient temperature, and the mixture was stirred for 24 h. Drying, filtering, and evaporating an organic phase yielded the compound after cleaning with water.

3. RESULTS AND DISCUSSION

In order to obtain maximum benefits from ALR2 inhibition, ARIs need to be highly specific and selective. Despite their strong affinity for the enzyme, the inhibitors that are currently on the market have a high level of nonspecific toxicity. Further studies must be conducted on ARIs so as to determine the extent and duration of the inhibition of the enzyme for a given dose and, more importantly, to study new ARIs that selectively reduce the enzymatic capacity so as to enhance the reduction of glutathione-conjugates (important for the transmission of intracellular signals) and glucose, without decreasing their detoxifying capacity towards aldehydes. The enzyme may have separate binding sites for glutathione and aldehydes, according to certain structure-activity studies using free aldehydes and their glutathione-conjugated analogues. Therefore, without compromising the enzyme's ability to detoxify aldehydes, specific alterations of the enzyme can stop it from recognizing and reducing glutathione-conjugates. These interesting findings suggest the likelihood of independently regulating the detoxifying role and the ability to transmit intracellular signals of ALR2.

By developing this important aspect, even more, selective inhibitors might be intended in the future, which could prevent cellular damage linked to diabetic complications without compromising the antioxidant defence. Numerous studies have established that ALR2 facilitates the aldehydes' reduction generated from lipids and their conjugates with glutathione, and protects against oxidative stress; therefore, a therapy based on the combination of ARIs and antioxidants could be effective. The beneficial and detoxifying effects of the enzyme decrease with the increase in the products of lipid peroxidation, and this can regulate the onset of long-term diabetic complications. The concomitant administration of ARIs and antioxidants, which can prevent peroxidation of lipids or enhance the antioxidant enzymes' expression, could recompense the reduced detoxifying action of ALR2. Antioxidant agents can also maintain the reduced enzyme form. Hence, one could avoid the development of typical ALR2 resistance towards drugs during hyperglycaemia, following its structural modifications [10].

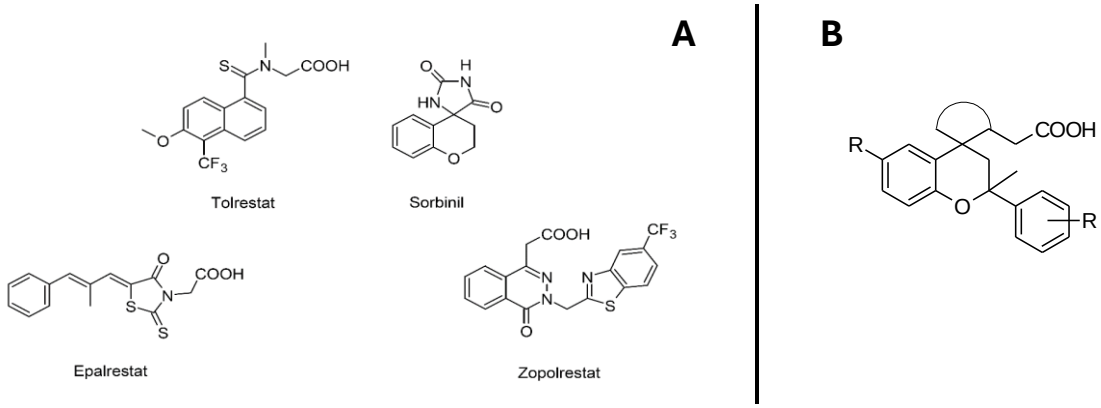

Figure 1. (**A**): Aldose reductase inhibitors: major carboxylic acid derivatives and cyclic imides. (**B**): Fusion of the classes of imides (sorbinil) and of the derivatives of acetic acid (tolrestat): possible resulting compounds

4. CONCLUSION

The chromatogram displays two peaks that can be attributed to the two di-stereoisomers of the spiro-oxalidinonic derivative analysed. The separation and evaluation of the components of the di-stereoisomeric mixture is important as it allows us to elucidate, in further studies, how the compounds' ability to inhibit ALR2 is affected by substitutions in positions 2 and 4 of the chromanonic system (Figure 1B).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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