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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Preliminary evaluation of rat kidney aldose reductase inhibitory activity of 2-phenylindole derivatives: affiliation to antioxidant activity

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Abstract Diabetic complications including nephropathy, neuropathy, and cataract are leading causes of end-stage renal diseases and neurological disorders. Aldose reductase (AR), the rate-limiting enzyme of the polyol pathway, catalyzes the reduction of glucose to sorbitol via NADPH. Excessive accumulation of intracellular sorbitol found in various tissues of diabetic animals and in cells cultured under high glucose conditions has been proposed to be an important factor for the pathogenesis of diabetic complications. Indole ring–containing AR inhibitors have received considerable attention as potential treatments for diabetic complications. However, these agents have not achieved worldwide use because of limited efficacy or unacceptable adverse effects. In this study, a series of 2-phenylindole derivatives were evaluated via an *in vitro* spectrophotometric assay for their ability to inhibit rat kidney AR. In addition, the antioxidant and AR inhibitory activities of the compounds under study were compared.

Keywords Aldose reductase · Antioxidant · Diabetes mellitus · Inhibitory · 2-Phenylindole

Introduction

Aldose reductase (AR) is member of the family of monomeric NADPH-dependent aldo–keto reductases. Along with sorbitol dehydrogenase, it makes up the polyol pathway (Spycher et al., 1997). In diabetes, the blood glucose level may increase

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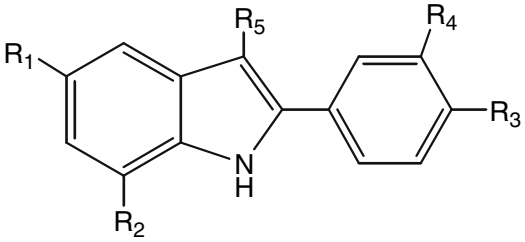
dramatically, which produces a marked rise in the glucose level in tissues such as nerve, lens, retina, kidney, and also in other tissues less involved in diabetic complications (Buyukbingol et al., 1994; Daş-Evcimen et al., 1998; Suzen and Buyukbingol, 2003). Despite having increasingly sophisticated treatment options for diabetes mellitus, diabetics often suffer from the eventual development of tissue-damaging complications which are largely responsible for the morbidity and mortality of diabetes. Experiments in diabetic animals have implicated sorbitol accumulation in the lens to the development of cataracts (Narayanan, 1993).

Recent efforts to identify treatments for chronic diabetic complications have resulted in the discovery of novel indole derivatives. Highly potent and selective 3-[(benzothiazol-2-yl)methyl]indole-*N*-alkanoic acid derivatives (van Zandt et al., 2005), cyano(2-oxo-2,3-dihydroindol-3-yl)acetic acid derivatives (da Settimo et al., 2003), (2-benzyl-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole-8-yl)-acetic acid (Djoubissie et al., 2006), [5-(4-pyrrol-1-yl-benzoyl)-1H-pyrrol-2-yl)]-acetic acid (Ananostou et al., 2002), 1-(6-hydroxyindole-1-yl)-2,2-dimethylpropan-1-one (Demopoulos et al., 2003), and 5-(3′-indolyl)-2-thiohydantoin (Buyukbingol et al., 1994) can be considered as some of the important examples. Recently it has been shown that diabetes is associated with increased oxidative stress (Spycher et al., 1997). The studies suggest that hydroxyl radical is indirectly inhibited by aldose reductase inhibitors (ARIs) resulting from decreasing polyol levels and hydroxyl radical formation. ARIs possessing antioxidant activity would therefore seem to be desirable. Oxidative stress plays a fundamental role in the pathogenesis of diabetes mellitus, particularly through progressive damage to proteins (Traverso et al., 1998, 1999).

Therefore, it is proposed that hydroxyl radical may accelerate damage to the cell membranes resulting from polyol accumulation (Suzen and Buyukbingol, 2003). Indole ring containing melatonin is a powerful antioxidant compound. It has been shown that melatonin increases antioxidant enzyme activity, inhibiting the release of superoxide radicals. A high total antioxidative capacity and the lower activity of AR increase melatonin scavenger capacity against reactive oxygen species in diabetic rats (Klepac et al., 2006). Melatonin also seems to be beneficial for diabetes therapy because of its potent nephroprotective action as well as antioxidative property. The indole-induced increase in the activities of the enzymes of glutathione metabolism might be important for antioxidative action of melatonin under diabetic conditions (Venkat Ratnam et al., 2006).

In our earlier study, we synthesized a series of 2-phenylindole (2PI) and 2-phenylindole-3-aldehyde (2PIA) derivatives (Table 1) (Suzen et al., 2006) to investigate their antioxidant activity. In this study, we investigated the ability of 2PI and 2PIA derivatives to inhibit rat kidney AR via an *in vitro* spectrophotometric assay. Some 2PI derivatives showed a strong scavenger effect on the superoxide anion. In addition, some of the compounds having a scavenging effect on the DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical were as effective as BHT (Butylated hydroxytoluene), a well known antioxidant utilized as positive control (Suzen *et al.*, 2006). These findings prompted us to screen and evaluate of 2PI derivatives as ARIs because of the relevance of diabetic complications such as cataract and free radical production (Hashim and Zarina, 2006).

Table 1 2-Phenyl indole derivatives tested for AR inhibition activity



Compound	R_1	R_2	R_3	R_4	R_5
1a	H	H	H	H	H
1b	H	H	F	H	H
1c	H	H	Cl	H	H
1d	H	H	Cl	Cl	H
1e	H	H	NH_2	H	H
1f	NO_2	NO_2	H	H	H
1g	H	H	Cl	NO_2	H
2a	H	H	H	H	CHO
2b	H	H	F	H	CHO
2c	H	H	Cl	H	CHO
2d	H	H	NH_2	H	CHO
2e	H	H	Cl	NO_2	CHO

Materials and Methods

We used male albino rats weighing 200–250 g and receiving a standard diet. Thirty rats were killed and kidney tissues were obtained. AR activity was determined after isolation from kidney tissue. All the enzyme experiments were performed in triplicate. Procedures involving the animals and their care conformed to institutional guidelines, in compliance with national and international laws and guidelines for the use of animals in biomedical research.

For the preparation of the 2PI derivatives, we used Fischer indole synthesis (Shiriner et al., 1955; Buyukbingol et al., 1994). Phenyl hydrazine and acetophenone derivatives were reacted to give hydrazones. Intramolecular cyclization of the hydrazones was performed using $ZnCl_2$. The 2PI derivatives 1a–g were then treated with $POCl_3$ and dimethyl formamide to give 2PI aldehydes 2a–f (Shabica et al., 1946) (Table 1). These compounds were synthesized and characterized by our research group to measure antioxidant activity (Suzen et al., 2006) and to determine electrochemical behavior in order to evaluate antioxidant activity mechanisms (Bozkaya et al., 2006).

Isolation of aldose reductase enzyme

The AR enzyme was isolated by a method (Cerelli et al., 1986) described below. Pooled kidney were thawed on ice and homogenized with 3 volumes of distilled water, followed by centrifugation at 10,000*g* for 20 min. Saturated ammonium sulfate was added to the supernatant to 40% saturation. The thick suspension was stirred for 15 min, followed by centrifugation at 10,000*g* for 20 min. The inert protein left in the supernatant was removed by increasing the ammonium sulfate concentration to 50% saturation followed by centrifuging the mixture at 10,000*g* for 20 min. The AR enzyme was precipitated from the 50% saturated solution by addition of powdered ammonium sulfate to 75% saturation and was recovered by centrifugation at 10,000*g* for 20 min. Protein concentration was measured by the method of Bradford (1976) using bovine serum as the standard.

Determination of aldose reductase activity

AR activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm via a UV-1700 Visible spectrophotometer. DL-Glyceraldehyde was used as the substrate. The enzyme was dissolved in 10 ml of 0.05 M NaCI solution and 0.1 ml was added to a quartz cuvette containing 0.1 ml of phosphate buffer (0.067 M, pH 6.2), 0.1 ml of NADPH (2×10^{-5} M final concentration), 0.1 ml of the test drug, and 2.4 ml of distilled water to obtain a 2.9-ml solution. The reaction is started by addition of 0.1 ml of DL-glyceraldehyde (5×10^{-5} M final concentration) to the cuvette, and the decrease in NADPH concentration was recorded at 340 nm for 5 min at 37 °C. Readings were taken at intervals in the periods when the changes in absorbance were linear. The results are shown in Table 2.

Results and Discussion

According to the *in vitro* AR inhibition results, compounds 1e, 1g, and 2a showed slight AR inhibitory activity but the following conclusions can be drawn from the results. Derivatives bearing *p*-NH_2 (1e) and *p*-Cl, *m*-NO_2 (1g) substituents on the phenyl ring at position 2 of the indole (2PI) are favorable for inhibition of the AR enzyme. While 2PI derivatives that have para or meta/para-Cl substituted phenyl ring in position 2 of the indole (1c, 1d) had no AR inhibition activity, addition of a *m*-NO_2 group to the molecule (1g) increased the activity. Interestingly, 2PIA derivatives with a CHO group in the third position of the indole ring exhibited no AR inhibitory activities; in fact these compounds showed some activation values. This finding clearly shows that an aldehyde function on the third position of indole ring (2PIA) definitely causes the decline in AR inhibition activity to zero. In our earlier study (Suzen et al., 2006), these 2PIA compounds showed no *in vitro* antioxidant activity while compounds 1e and 1g showed good antioxidant activity in two experiments, i.e., DPPH (free radical scavenging activity) via the Blois (1958) method and superoxide anion scavenging activity via the McCord and Fridowich

Table 2 Relationship between AR inhibition and antioxidant activity of 2-phenyl indole derivatives

Compound	AR inhibition (%)	Superoxide anion ($O_2^{\bullet -}$) scavenging activity at 10^{-3} M and 10^{-4} M concentrations[a]	DPPH free radical scavenging activity at 10^{-3} M and 10^{-4} M concentrations[a]
1a	No activity	Not significant	Not significant
1b	No activity	Less activity	Not significant
1c	No activity	Less activity	Not significant
1d	No activity	Moderate activity	Not significant
1e	11.00 ± 6.00	Moderate activity	Strong activity
1f	No activity	Strong activity	Strong activity
1g	5.29 ± 2.14	Strong activity	Moderate activity
2a	7.64 ± 4.25	No activity	No activity
2b	No activity	No activity	No activity
2c	No activity	No activity	No activity
2d	No activity	No activity	No activity
2e	No activity	No activity	No activity

Values represent the mean ± SD of three individual experiments

[a] Data from Suzen et al. (2006)

(1969) method as seen in Table 2. There is a good correlation between these *in vitro* antioxidant experiments results and *in vitro* AR inhibition results of 2PI derivatives.

Diseases associated with oxidative stress such as diabetes mellitus and cancer show a pro-oxidative shift in the redox state and impaired glucose clearance, suggesting that muscle mitochondria is the major site of elevated ROS (Reactive Oxygen species) production. This condition may be referred to as ''mitochondrial oxidative stress.'' The increased ROS production or changes in intracellular glutathione levels are often involved in pathological changes (Droge, 2002: Waris and Ahsan, 2006).

Known inhibitors of AR vary widely in structure, and some of them exhibit strong species specificity. It is apparent that specificity for binding at the inhibitory site of AR must eventually depend on the hydrophilicity, electronic environment, and conformational properties of individual inhibitors (Suzen and Buyukbingol, 2003).

In summary, we have reported preliminary *in vitro* biological evaluation of substituted 2PI and 2PIA derivatives as AR inhibitors. Compounds 1e, 1g (2PI), and 2a (2PIA) demonstrated some AR inhibition activity. Although the results are not significant, a correlation may be drawn between these compounds and their antioxidant activities (DPPH antiradical activity and superoxide radical scavenging activity). The results of the biological evaluation allowed us to obtain insight into the initial structural features critical for AR inhibition in this series. Thus, based on these findings, further modifications on the present series of compounds are needed. The search for new ARIs possessing more favorable biological properties is still a major pharmaceutical challenge (Pau et al., 2004).

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