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RESEARCH ARTICLE

Rat Lung Aldose Reductase Inhibition Capacity of Substituted Indole Hydrazide/Hydrazone Derivatives

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SUMMARY

Diabetes Mellitus is one of the most serious health problem facing both developed and developing countries. Long term complications lead to morbidity and mortality in patients with diabetes. Increased polyol pathway has been implicated in the pathogenesis of microvascular complications and cataract. Aldose reductase (AR) is the key enzyme of the polyol pathway, which converts glucose to sorbitol. Excessive accumulation of sorbitol is associated to the diabetic complications. Avoidance of sorbitol accumulation by inhibiting AR would be an efficient treatment. Due to the significance of AR as a potential drug target in the treatment of diabetic complications, there are increasing interests in the design and synthesis of AR inhibitors. In this study, 2-fluorophenylindol and 5-chloroindole hydrazide/hydrazone derivatives were tested for measuring the AR enzyme inhibitory activity. The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation, which accompanies the reduction of D,L-glyceraldehyde used as substrate. Results showed in general 52 - 60 % inhibitory activity in halogen substituted indole derivatives. This study proposes a new approach for the in vitro AR inhibition activity properties and structure activity relationship of 2, 3- and 5-substituted indole ring. For the inhibitory activity, not only the indole ring is important, but also is the side chain containing the amide group and halogens.

Key Words: *Aldose reductase, diabetes, polyol pathway, indole, inhibition.*

Sübstitüe İndol Hidrazit/Hidrazon Türevlerinin Rat Akciğer Aldoz Redüktazını İnhibe Etme Kapasiteleri.

ÖZET

Diyabet gelişmiş ve gelişmekte olan ülkelerde önemli sağlık sorunlarından biridir. Diyabetli hastalarda uzun süren komplikasyonlar sağlık sorunlarına ve ölüme neden olmaktadır. Poliyol yolağının aktivitesindeki artış mikrovasküler komplikasyonlara ve katarakta neden olmaktadır. Aldoz redüktaz (AR) glukozu sorbitole çeviren poliyol yolağının anahtar enzimidir. Sorbitolun aşırı birikimi diyabetik komplikasyonlara neden olmaktadır. AR inhibitörleri ile sorbitol birikiminin önlenmesi en etkili tedavi yöntemlerinden biri olabilir. Diyabetik komplikasyonların tedavisinde AR'ın potansiyel ilaç hedefi olmasına bağlı olarak, AR inhibitörlerinin tasarımı ve sentezine ilgi artmıştır. Bu çalışmada, 2-fulorofenilindol ve 5-kloroindol hidrazit /hidrazon türevlerinin AR enzimi inhibisyon aktiviteleri ölçülmüştür. Enzim aktivitesi NADPH oksidasyonunu takip ederek spektrofotometrik yöntem ile tayin edilmiştir. D,L-gliseraldehit substrat olarak kullanılmıştır. Sonuç olarak, halojen substitue indol türevleri genel olarak % 52-60 oranında inhibitör aktivite göstermektedir. Bu çalışma, 2,3- ve 5-sübstitüe indol halkasının in vitro AR inhibisyonu özelliği ve yapı aktivite ilişkisi açısından yeni bir bakış açısı sunmaktadır. İnhibitör aktivite için sadece indol halkası değil aynı zamanda amid grubu ve halojenleri içeren yan zincirinde önemi vardır.

Anahtar kelimeler: *Aldoz Redüktaz, Diyabet, Poliyol Yolağı, İndol İnhibisyonu*

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, and diabetic complications lead to morbidity and death in diabetic patients. Hyperglycemia has been shown to be the most important risk factor in charge for the systemic complications. Various biochemical pathways have been proposed to explain the adverse effects of hyperglycemia. Potential cellular mechanisms of hyperglycemia-induced complications are the activation of diacylglycerol-protein kinase C, increased polyol pathway, enhanced reactive oxygen species production, nonenzymatic glycation and advanced glycation end products pathway (1-4).

Aldose reductase (AR) is a protein, belonging to the aldo–keto reductase superfamily (5) that catalyzes

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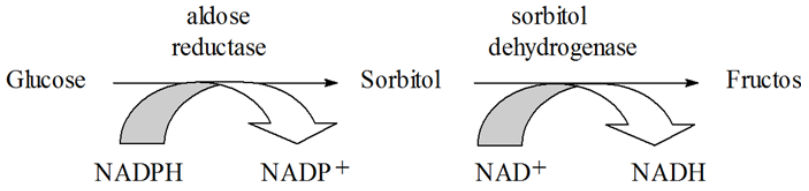

Figure 1. Polyol pathway.

the first step of the polyol pathway (Fig. 1). AR has been demonstrated to play an important role in the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy (2). The role of polyol pathway in diabetic complications may have different mechanisms, such as; accumulation of sorbitol or fructose (6, 7), myo-inositol depletion (8), or alterations in NADPH/NADP and NADH/ NAD ratios (9, 10).

AR enzyme not only reduces glucose to sorbitol, but also reduces a broad variety of aldehydes and their conjugates with glutathione (11). Kinetic and structural studies suggest that under normoglycemic conditions, reduction of glucose may have a secondary role of AR (12). The preferred substrates of the enzyme are aromatic aldehydes and medium- to long-chain aliphatic aldehydes derived from lipid peroxidation. AR has a valuable role in the detoxification of toxic lipid aldehydes upon oxidative stress (11).

Investigational studies indicated that AR inhibitors (ARIs) also affected the oxidative stress (13-15). Studies have shown that ARIs reduce the lipid hydroperoxides in diabetes (16) and detoxify the reactive carbonyl compounds derived from oxidative stress (17).

Prevention of sorbitol accumulation by inhibiting the AR activity would be an effective treatment of diabetic complications (5,18). However, none of the presently available treatments emerge to achieve the necessary prevention of the development of diabetic complications in diabetic patients. Because of shortage of drugs currently available for the treatment diabetic complications, the search for new ARIs with more favorable biological properties is still a major pharmaceutical challenge. It is known that developing an efficient drug against AR enzyme can be possible with innovative strategies by focusing on rational design of chemical entities able to affect simultaneously multiple key mechanisms (19). There are many successful research related with inhibition of AR with indole derivatives such as indole acetic acids and tetrahydropyridoindole (19, 20), pyridoindoles (21, 22) and indole-N-alkanoic acids (23). In our earlier studies AR inhibition with indole derivatives showed noteworthy results (24-29). In light of these findings, thirty six indole hydrazide/ hydrazone derivatives (Fig. 2) were evaluated via an in vitro spectrophotometric assay for their ability to inhibit rat lung AR.

MATERIALS AND METHODS

Chemistry

The target imines derived from 5-chloro-1*H*-indole-3-carboxaldehyde or 2-flourophenyl-1*H*-indole-3-carboxaldehyde and appropriate hydrazine or hydrazide derivatives in the presence of ethanol. Finally, N,N'-bis-indole derivatives was synthesized using equimolar amounts of hydrazine hydrate with 5-chloro-1*H*-indole-3-carboxaldehyde in the presence of ethanol. All the compounds were characterized on the basis of spectral data and published earlier (28, 29).

Animals

Male Albino rats weighing 200–250 g were used for experiments. They received standard diet. 10 rats were killed and lung tissues were discarded. AR enzyme was isolated from the lung tissues and the enzyme activity was determined following the isolation. All the enzyme experiments were performed in triplicate. Procedures involving the animals were cared for in accordance with the principles of the "Canadian Council on animal Care Guide to the Care and Use of Experimental Animals". The studies were approved by the animal experiment local ethics committee, Ankara University. All procedures were performed consistently by the same investigator.

Isolation of aldose reductase enzyme

The AR enzyme was isolated by a method (30) described below. Pooled lung were thawed on ice and homogenized with 3 volume of distilled water, followed by centrifugation at 10,000xg for 20 min saturated ammonium sulfate was added to the supernatant to 40% saturation. The thick suspension had been stirred for 15 min, followed by centrifugation at 10,000xg 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 10,000xg for 20 min. The AR enzyme was precipitated from the 50% saturated solution by adding powdered ammonium sulfate to 75% saturation and was recovered

by centrifugation at 10,000xg for 20 min. Protein concentration was measured by the method of Bradford (31) 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 by a UV-1700 Visible spectrophotometer (30). DL-glyceraldehyde was used as a substrate. The enzyme was dissolved in 10 ml 0.05 M NaCl solution. 0.50 mg protein was added to a quartz cuvette containing 0.6 ml phosphate buffer (0.067 M, pH 6.2), 0.1 ml NADPH ($2{,}5x10^{-4}$ M final concentration), $1x10^{-5}$ M of the test drug (final concentration) (solutions prepared in 50% DMF–50% methanol). Reaction was started by the addition of 0.1 ml DL-glyceraldehyde ($1x10^{-2}$ M final concentration) to the cuvette and the decrease in NADPH concentration was recorded at 340 nm for 5 min at 37^0C. Readings were taken at intervals in the periods when the changes in absorbance were linear. The results are shown in Table 1.

Table 1. Results of aldose reductase inhibition by indole hydrazide / hydrazone derivatives

Compound	%Inhibition	Compound	%Inhibition
1	0.00 ± 0.00	19	**33.33 ± 6.35**
2	3.33 ± 1.73	20	7.94 ± 3.65
3	0.00 ± 0.53	21	4.76 ± 2.46
4	6.67 ± 2.47	22	11.91 ± 2.06
5	5.33 ± 3.00	23	2.65 ± 1.06
6	14.00 ± 3.53	24	1.99 ± 3.05
7	22.67 ± 2.87	25	7.95 ± 0.79
8	**52.67 ± 2.07**	26	18.54 ± 0.93
9	23.33 ± 1.93	27	5.96 ± 3.51
10	24.00 ± 1.47	28	4.64 ± 0.79
11	21.33 ± 1.40	29	12.58 ± 0.00
12	16.67 ± 0.87	30	9.27 ± 2.25
13	52.67 ± 3.00	31	17.88 ± 3.91
14	60.00 ± 3.40	32	1.99 ± 1.66
15	3.17 ± 0.37	33	15.89 ± 0.33
16	0.00 ± 0.00	34	8.61 ± 1.72
17	3.17 ± 2.06	35	15.89 ± 3.51
18	0.00 ± 0.00	36	21.19 ± 0.33

Values represent the mean ± S. D. of three individual experiments.

Statistics

All analyses were performed in triplicate and the data were calculated as means ± Standard Deviations.

RESULTS AND DISCUSSION

In this study, 36 indole hydrazide/hydrazone derivatives (Fig. 2) were evaluated for their ability to inhibit rat lung AR by an in vitro spectrophotometric assay. The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation, which accompanied the reduction of D,L-glyceraldehyde used as substrate. The inhibition study was performed merely by using 10^{-5} M concentration in which no additional study seemed to be necessary to obtain IC_{50} values. Within indole derivatives **14** was shown to have the highest inhibitory effect. This was followed by **13**, **8** and **19** with the 52.67 %, 52.67 % and 33.33 % inhibition activities respectively. The rest of the compounds had no significant inhibition potency at 10^{-5} M concentration. Sorbinil was used as a positive control which is the most investigated specific aldose reductase inhibitor. 10^{-5} M sorbinil has shown 82.46 % inhibition activity in our study. Compound **14** which had anisic acid and florophenyl side chains on the indole ring showed the highest AR inhibition rates. However compound **35** which had the anisic acid but no florophenyl side chain showed only 15,89 % inhibition. This indicates that these two side chains are necessary for the maximum activity and also florophenyl is essential for the AR inhibitory activity. Compounds **8** and **13** showed 52.67 % inhibition rate and they contained o-bromophenyl and diflorophenyl side chains. In general, Br substituted derivatives were found to be more active than the rest of the compounds. This was followed by F substituted derivatives. This is noteworthy as ARIs ponalrestat, minalrestat, zenarestat and ranirestat have bromo-fluorobenzyl group as side chain to main ring system. Results of this study emphasized the necessity of the halogens especially Br and Cl for the AR inhibitory activity. However AR inhibition data clearly showed that introduction of a p-florophenyl group on the second position of indole ring led to a marked decrease in AR inhibitory potency. Moreover, contradictory to the 5-chloroindole derivatives' introduction of halogenated phenyl side chain on 5^{th} position of indole ring did not improve the AR inhibitory activity in this series of compounds.

CONCLUSION

ARIs are one of quite a few types of drugs that have shown prevention of diabetic complications (32, 33). It is still a challenge to develop a candidate drug. Thus AR has long been recognized as an important target for preventing diabetic complications (34). At present, Epalrestat is the only ARI available on the market, and the research area requires further work. On the basis of our preliminary AR inhibitory screening results on indole derivatives, we embarked on the synthesis of more derivatives to discover more active molecules.

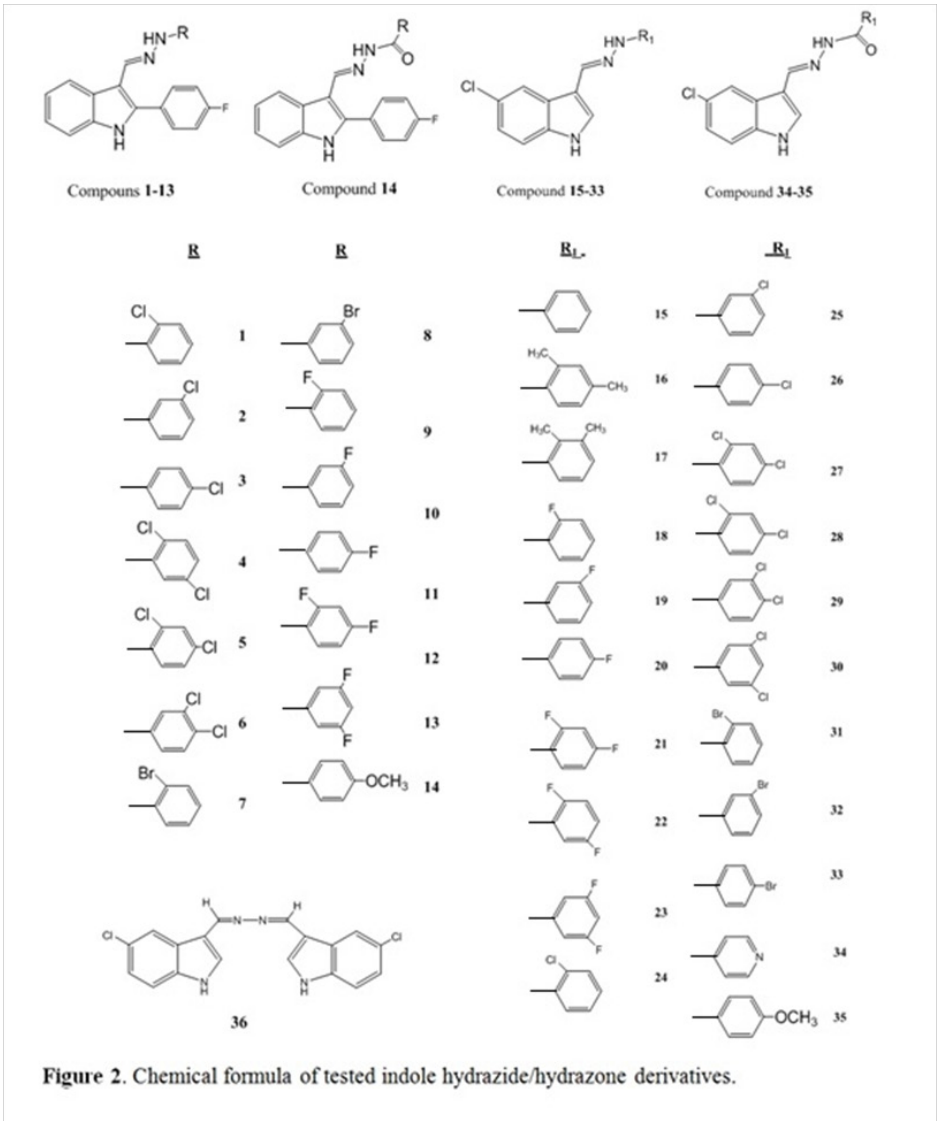

Figure 2. Chemical formula of tested indole hydrazide/hydrazone derivatives.

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