https://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 30, Number 1, Pages 13-24 (2021)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/58411/798355

Received by the Editors: October 20, 2020; Accepted: December 14, 2020

ALTERATIONS OF ALDOSE REDUCTASE ACTIVITY BY INDOLE-3-CARBOXALDEHYDE DERIVATIVES

Orçun Erdem KURŞUN[1], Sibel SÜZEN[2], and Özlem YILDIRIM[1]

[1]Ankara University, Faculty of Sciences, Department of Biology, 06100 Tandoğan, Ankara, TURKEY,
[2]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Tandoğan, Ankara, TURKEY

ABSTRACT. In diabetic conditions, aldose reductase (AR, EC 1.1.1.21) activity is significantly increased in lens, kidney and nerve tissues. Mainly in ocular and neural tissues increased levels of sorbitol is associated with diabetic complications such as retinopathy, nephropathy, neuropahty, cataract formation and also tissue damage via increased reactive oxygen speices. Recently, there are many studies that show the relationship between this enzyme family and cancer. Aldose reductase is important for the pathway and in turn has been a potential target for drug design. Great number of aldose reductase inhibitors (ARIs) are used for prevention or delay of these diabetic complications and cancer. However, effective ARIs which has benefits in diabetic complications are still under investigation. In this study aldose reductase was partially purified from bovine lens and the inhibitory effects of 16 different indol-3-carboxyaldehyde derivatives on aldose reductase enzyme activities were examined by kinetic assays. These results suggested that *N*'-[(5-bromo-1*H*-indol-3-yl)methylidene]pyridine-4-carbohydrazide showed that the highest inhibitory activity on AR.

Aldose reductase (AR; EC 1.1.1.21; AKR1B1, ALD2) is the major enzyme in polyol pathway which is associated with abnormal glucose metabolism and diabetic complications [1]. There are many reports implying critical roles for AR in accelerating atherosclerosis, mediating ischemic myocardial and vascular injury in diabetes, aging, cardiomyopathy, hypertension etc. [2-5]. AR is characterized with hyperglycemia which cause to development of secondary diabetic complications that microvascular (cataract, diabetic retinopathy, neuropathy and nephropathy) and

Keyword and phrases. Aldose reductase, inhibition, diabetes mellitus, indole derivates.

oekursun@hotmail.com; sibel@pharmacy.ankara.edu.tr; Corresponding author: yildirim@science.ankara.edu.tr
0000-0001-5385-7989; 0000-0003-3413-6152; 0000-0003-1018-0335

macrovascular (cardiovascular, peripheral arterial and cerebrovascular) diseases [2-5].
Furthermore, some researches indicated that there is overexpression of AR in several human cancer tissues such as breast, cervix, ovary and rectum [6,7]. AR mediates oxidative stress-induced signals that increase inflammatory products in the vasculature of organs [8,9]. This might trigger the angiogenic process in cancer endothelial cells [10], which play a significant role in apoptosis, cell proliferation, metastasis and angiogenesis [11]. Several experimental data propose that inhibition of AR can prohibit ROS which produced by growth factors-, chemokines-, cytokines- and hyperglycemia via inhibition of redox-sensitive transcriptions factors, NF-κB and AP-1 activation [12,13]. AR inhibitors (ARIs) can provide therapeutic potential in cardiovascular diseases associated with diabetes, myocardial injury, aging and can prevent cancer growth and metastasis [14-16]. ARIs have been shown to anticipate the progress of retinopathy, cataracts, neuropathy and nephropathy. Therefore, these complications develop the idea of improving several ARIs [17,18].

ARIs are divided into two groups such as; carboxylic acid and hydantoin [19]. These sub-groups are known to interact with the hydrophilic and hydrophobic sites of AR [20-23]. Effects of some indole derivatives on AR are studied to prevent and/or delay the formation of cataract and other complications also effecting on the various cancer tissues and being alternative to the other ARI's. Miyamoto [24] has found that an indole acetic acid derivative showed more than 40% inhibition of AR at a concentration of 15 μg/mL. After subsequent lead optimization study based on the predicted docking mode, approximately 20-fold increase was achieved in inhibitory activity of the indole derivative. A large range of structurally different compounds have been studied as potential *in vitro* ARIs [24-27]. However, with few exceptions, these compounds did not show a significant clinical benefit.

Epalrestat was developed in 1983 [28] and this is the only ARI approved as a therapeutic drug for clinical treatment of diabetic neuropathy. Tolrestat was improved as a strong ARI, however it was withdrawn for the reason of liver toxicity [29]. Previous research showed that carboxylic acid containing ARIs zenarestat [30], zopolrestat [31], and ponalrestat [32] have renal toxicity and they were withdrawn from clinical trials due to lack of efficacy [33-35]. Lidorestat has poor tissue penetration [36]. So far only fidarestat and ranirestat have been recognized as potential ARIs in diabetic complications [37].

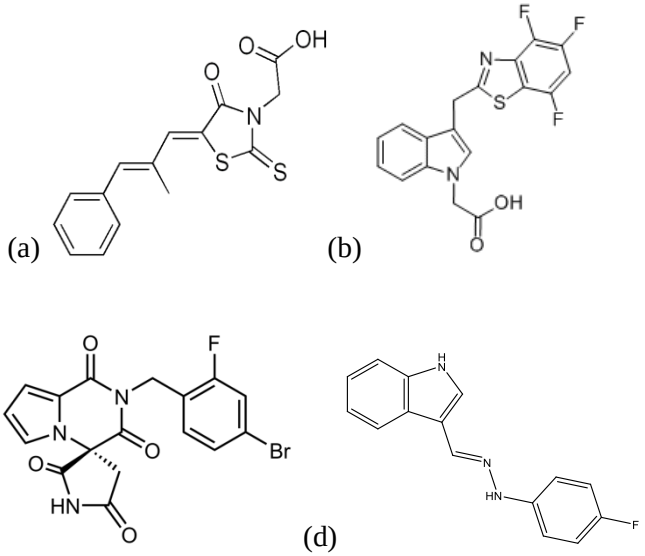


FIGURE 1. Epalrestat (a), lidorestat (b), ranirestat (c) andsynthesisedindole-3-phenylhydrazinylidene derivative (d) [40].

We have been studying on the ARIs and the antioxidant properties of indole derivative compounds for more than a decade [25, 38-41]. In this study, the effects of indol-3-carboxaldehyde derivatives which were described previously [42] were examined for the inhibition of bovine lens AR *in vitro*. Moreover, the new substitute indole derivatives was designed as the inhibitor of AKR1B1. Sixteen indole derivatives (including indole-3- phenylhydrazinylidene, of indole-3-ylmethylidene and indole-3,3'- bis(1*H*-indole) derivatives) were synthesized and evaluated to understand the AR inhibitor capacities.

2. MATERIALS AND METHODS

2.1. Subjects

Sixty bovine lenses were used for experiments. They were obtained from Haymana slaughterhouse and received standard diet. Bovine eyes were frozen at -80 °C. AR enzyme was isolated from the lens tissues and the enzyme activity was determined with the following isolation method. All the enzyme experiments were performed in triplicate. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in European Community guidelines.

2.2. Isolation of Aldose Reductase Enzyme from Bovine Lens

Sixty bovine lenses were thawed on ice and homogenized with 3 volumes of distilled water. Homogenate were centrifuged at 10,000 X g for 20 min as indicated in Schema 1 [39, 43]. At the end of last centrifugation (as indicated in schema) the pellet was collected and dissolved in 0.05M NaCl before use for enzyme assay.

2.3. Determination of protein concentration

Protein concentration was measured by the method of Bradford [44] using bovine serum albumin as a standard.

2.4. Determination of Aldose Reductase Activity

AR enzyme activity of the freshly prepared supernatant was assayed spectrophotometrically. The activity was determined by the decrease in NADPH concentration at 340 nm by a UV-1700 Visible spectrophotometer [39,45]. The enzyme was dissolved in 5 ml 0,05 M NaCI solution. Then 0,1 ml of this solution was added to a quartz cuvette which contains 0.1 ml phosphate buffer (0,067 M, pH:6,2), 0,1ml NADPH (2×10^{-5} M final concentration), 0,1 ml of the test drug (10^{-4} M solutions prepared in 50 % DMF and 50 % methanol) and 2,5 ml distilled water to obtain final volume 2,9 ml solution. The reaction was initiated by the adding of 0,1 ml 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 ^{o}C. The readings were taken at the intervals when the changes in absorbance were linear. The results represent three individual experiments.

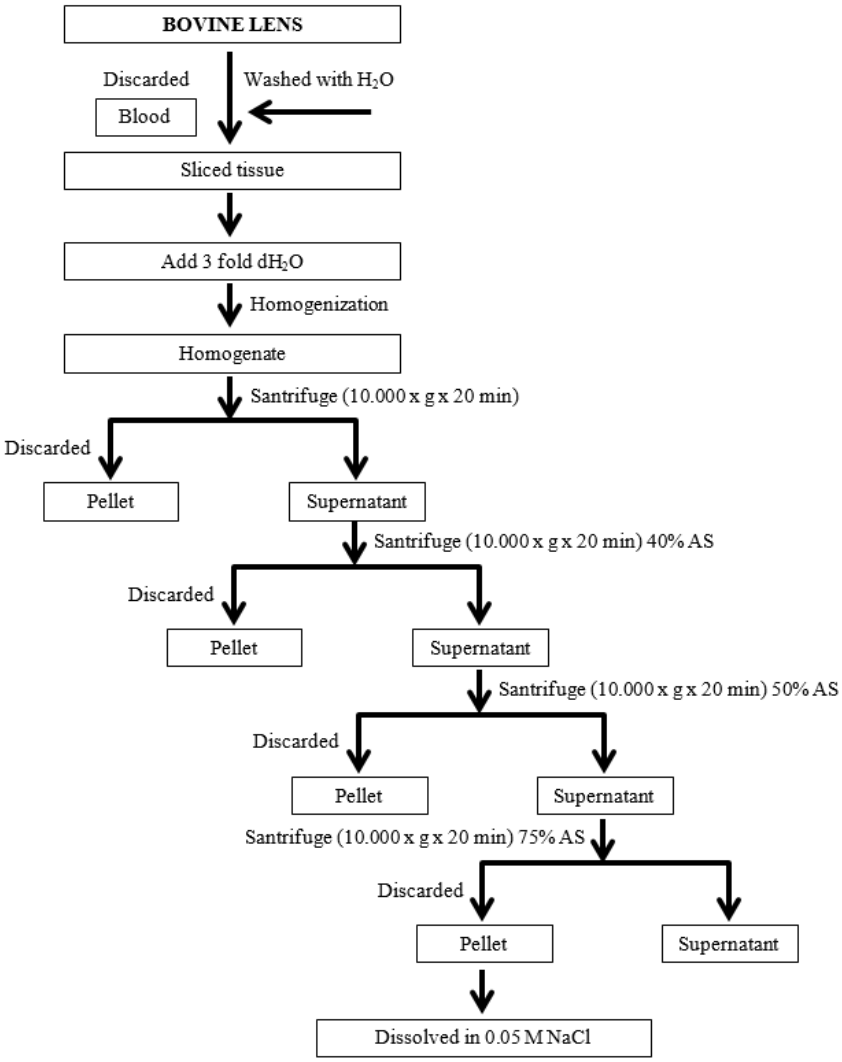


FIGURE 2. Schematic presentation of AR isolation from bovine lens.

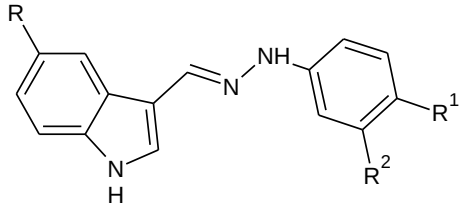
3. RESULTS AND DISCUSSION

The *in vitro* AR inhibition results obtained by indole derivatives are represented in Table 1, 2 and 3. The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation which accompanies the reduction of D-L-

glyceraldehyde substrate. The inhibition study was performed at 10^{-4} M concentration of each compound.

The highest inhibition values were found in compounds 7, 10 and 14 with inhibition 25.49 ± 5.01 %, 23.08 ± 10.99 % and 43.0 ± 3.49 % respectively. These three compounds have Br atom on the 5th position of the indole ring. Surprisingly, compound 2 which does not contain Br on the indole ring but it contains Br atom on the phenyl side chain. However, compound 2 did not show inhibition. These results clearly indicated that the halogen substitution on the indole ring increased inhibition.

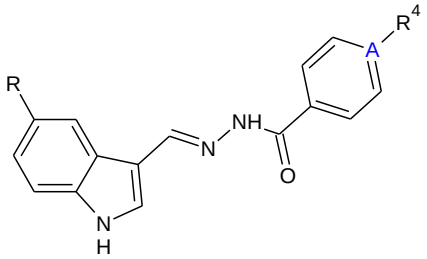
TABLE 1. Bovine lens AR inhibition activity of indole-3- phenylhydrazinylidene derivatives



Compounds		R	R^1	R^2	Inhibition (%)
1	3-[(2-phenylhydrazinylidene)methyl]-1*H*-indole	H	H	H	No inhibition
2	3-{[2-(4-bromophenyl)hydrazinylidene] methyl}-1*H*-indole	H	Br	H	No inhibition
3	3-{[2-(4 fluorophenyl)hydrazinylidene]methyl}-1*H*-indole	H	F	H	3.42 ± 2.59
4	3-{[2-(4-chlorophenyl)hydrazinylidene] methyl}-1*H*-indole	H	Cl	H	No inhibition
5	3-{[2-(3,4-dichlorophenyl)hydrazinylidene] methyl}-1*H*-indole	H	Cl	Cl	No inhibition
6	5-Bromo-3-[(2-phenylhydrazinylidene)methyl]-1*H*-indole	Br	H	H	No inhibition
7	5-Bromo-3-{[2-(4-bromophenyl) hydrazinylidene] methyl}-1*H*-indole	Br	Br	H	25.49 ± 5.01

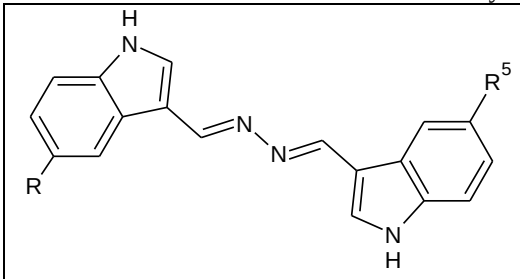
8	5-Bromo-3-{[2-(4-fluorophenyl) hydrazinylidene]methyl}-1*H*-indole	Br	F	H	No inhibition
9	5-Bromo-3-{[2-(4-chlorophenyl)hydrazinylidene]methyl}-1*H*-indole	Br	Cl	H	0.43 ± 11.51
10	5-Bromo-3-{[2-(3,4-dichlorophenyl)hydrazinylidene]methyl}-1*H*-indole	Br	Cl	Cl	23.08 ± 10.99

TABLE 2. Bovine lens AR inhibition activity of indole-3-ylmethylidene derivatives



Compounds		R	R^4	A	Inhibition (%)
11	*N*'-[1*H*-indol-3-ylmethylidene]-4-methoxybenzohydrazide	H	OCH_3	C	No inhibition
12	*N*'-[1*H*-indol-3-ylmethylidene]pyridine-4-carbohydrazide	H	-	N	2.16 ± 6.05
13	*N*'-[(5-bromo-1*H*-indol-3-yl)methylidene]-4-methoxybenzohydrazide	Br	OCH_3	C	No inhibition
14	*N*'-[(5-bromo-1*H*-indol-3-yl)methylidene]pyridine-4-carbohydrazide	Br	-	N	43 ± 3.49

Table 3. Bovine lens AR inhibition activity of indole-3,3'- bis(1*H*-indole) derivatives



Compounds		R	R^5	Inhibition (%)
15	3,3'-[hydrazine-1,2-diylidenedimethylylidene]bis(1*H*-indole)	H	H	No inhibition
16	3,3'-[hydrazine-1,2-diylidenedimethylylidene]bis(5-bromo-1*H*-indole)	Br	Br	No inhibition

Compounds which have izonicotinoyl hydrazone side chains (compounds 12 and 14) showed interesting results. Compound 14 which is a 5 bromo derivative showed the highest inhibitory result as 43.0 ± 3.49 % while compound 12 has very slight inhibition rate as 2.16 ± 6.05%.

Research showed that three regions play important role on the AR for the interaction with drug molecules. These are basically a substrate site, a nucleotide fold, and an inhibitor site (2). The compounds have possessed the essential structural requisites (an acidic proton, hydrogen-bond acceptor groups and an aromatic moiety) for AR inhibition (32, 8). In our study, most likely the compounds which has more electronegative property lead to establishment of hydrogen bonds with side groups of Tyr, His and Trp amino acids at active site of AR. Also, aromatic-aromatic interactions establishment between the aromatic groups at active site and at the synthesized compounds increased the inhibition. Drug development studies on inhibiting AR requires further experiments on analyzing the structure-activity relationships of the synthesized compounds to reach a target molecule.

4. Conclusion

AR activity significantly increases in several tissues in diabetic conditions. Consequently, ARIs are main therapeutic targets in prevention and delaying progression of several diseases including diabetes and cancer. Indole-3-phenylhydrazinylidene, indole-3-ylmethylidene and indole 3-3'-bis(1*H*-indole) derivatives have been screened for their AR inhibitory activities. *N*'-[(5-bromo-1*H*-indol-3-yl)methylidene]pyridine-4-carbohydrazide showed the highest inhibitory activity against bovine AR enzyme. Further experimental analysis is necessary to reveal the inhibition mechanisms of the synthesized molecules and their beneficial effects.

References

[1] Brownlee, M., Biochemistry and molecular cell biology of diabetic complications, *Nature*, 414 (2001), 813-20.

[2] Balasubramaniam, M., Rema, M., Premanand, C., Biochemical and molecular mechanisms of diabetic retinopathy, *Science*, 83 (2002), 1506-14.

[3] Srivastava, S.K., Ramana, K.V., Bhatnagar, A., Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options, *Endocr Rev*, 26 (2005), 380-92.

[4] King, K.D., Jones, J.D., Warthen, J., Microvascular and macrovascular complication in Diabetes Mellitus, *Am J Pharm Educ*, 69 (2005), 1-10.

[5] Vedantham, S., Ananthakrishnan, R., Schmidt, A.M., Ramasamy, R., Aldose reductase, oxidative stress and diabetic cardiovascular complications, *Cardiovasc Hematol Agents Med Chem*, 10 (2012), 234–40.

[6] Saraswat, M., Mrudula, T., Kumar, P.U., Suneetha, A., Rao, T.S., Srinivasulu, M., Reddy, G.B., Overexpression of aldose reductase in human cancer tissues, *Med Scien Monitor*, 12 (2006), 525-29.

[7] Tammali, R., Reddy, A.B., Srivastava, S.K., Ramana, K.V., Targeting Aldose Reductase for the Treatment of Cancer, *Curr Cancer Drug Targets*, 11(5) (2011), 560-571.

[8] Tammali, R., Reddy, A.B., Srivastava, S.K., Ramana, K.V., Inhibition of Aldose Reductase Prevents Angiogenesis in vitro and in vivo, *Angiogenesis*, 14 (2011), 209-21.

[9] Milackova, I., Prnova, M.S., Majekova, M., Sotnikova, R., Stasko, M., Kovacikova, L., Banerjee, S., Veverka, M., Stefek, M., 2-Chloro-1,4-naphthoquinone derivative of quercetin as an inhibitor of aldose reductase and anti-inflammatory agent, *J Enz Inhib Med Chem*, 30 (2015), 107-13.

[10] Reuter, S., Gupta, S.C., Chaturvedi, M.M., Aggarwal, B.B., Oxidative stress,

inflammation, and cancer: How are they linked?, *Free Radic Biol Med*, 49 (2010), 1603–1616.

[11] Maccari, R., Vitale, R.M., Ottanà, R., Rocchiccioli, M., Marrazzo, A., Cardile, V., Graziano, A.C., Amodeo, P., Mura, U., Del Corso, A., Structure-activity relationships and molecular modelling of new 5-Arylidene-4-thiazolidinone derivatives as aldose reductase inhibitors and potential anti-inflammatory agents, *Eur J Med Chem*, 81 (2014), 1−14.

[12] Ramana, K.V., Bhatnagar, A., Srivastava, S., Yadav, U.C., Awasthi, S., Awasthi, Y.C., Srivastava, S.K., Mitogenic responses of vascular smooth muscle cells to lipid peroxidation-derived aldehyde 4-hydroxy-trans-2-nonenal (HNE): role of aldose reductase-catalyzed reduction of the HNEglutathione conjugates in regulating cell growth, *J Biol Chem*, 281 (2006), 17652–17660.

[13] Tammali, R., Ramana, K.V., Srivastava, S.K., Aldose reductase regulates TNF-alpha-induced PGE2 production in human colon cancer cells, *Cancer Lett*, 252 (2007), 299–306.

[14] Zeng, K.W., Li J., Dong, X., Wang, Y.H., Ma, Z.Z., Jiang, Y., Jin, H.W., Tu, P.F., Anti Neuroinflammatory Efficacy of the Aldose Reductase Inhibitor FMHM via Phospholipase C/Protein Kinase C Dependent NF-κB and MAPK Pathways, *Toxicol Appl Pharmacol*, 273 (2013), 159−171.

[15] Shukla, K., Sonowal, H., Saxena, A., Ramana, K.V., Srivastava, S.K., Aldose Reductase Inhibitor, Fidarestat Regulates Mitochondrial Biogenesis Via Nrf2/HO-1/AMPK Pathway In Colon Cancer Cells, *Cancer Lett*, 411 (2017), 57-63.

[16] Huang, Q., Liu, Q., Ouyang, D., Sorbinil, an Aldolase Reductase Inhibitor, in Fighting Against Diabetic Complications, *Med Chem*, 15 (1) (2019), 3-7.

[17] Chang, K.C., Laffin, B., Ponder, J., Énzsöly, A., Németh, J., LaBarbera, D.V., Petrash, J.M., Beta-Glucogallin Reduces the Expression of Lipopolysaccharide-Induced Inflammatory Markers by Inhibition of Aldose Reductase in Murine Macrophages and Ocular Tissues, *Chem Biol Interact*, 202 (2013), 283−287.

[18] Li, L., Chang, K.C., Zhou, Y., Shieh, B., Ponder, J., Abraham, A.D., Ali, H., Snow, A., Petrash, J.M., LaBarbera, D.V., Design of an Amide N-Glycoside Derivative of β-Glucogallin: A Stable, Potent, and Specific Inhibitor of Aldose Reductase, *J Med Chem*, 57 (2014), 71−77.

[19] Shoeb, M., Ramana, K.V., Srivastava, S.K., Aldose Reductase Inhibition Enhances TRAIL-Induced Human Colon Cancer Cell Apoptosis through AKT/FOXO3a-dependent Upregulation of Death Receptors, *Free Radical Biol Med*, 63 (2013), 280−290.

[20] Suzen, S., Buyukbingol, E., Recent studies of aldose reductase enzyme inhibition for diabetic complications, *Curr Med Chem*, 10 (2003), 1329-52.

[21] El-Kabbani, O., Ruiz, F., Darmanin, C., Chung, R.P.T., Aldose reductase structures: implications for mechanism and inhibition, *Cell Mol Life Scien*, 61 (2004), 750-62.

[22] Sun, G., Ma, Y., Gao, X., König, S., Fales, H.M., Kador, P.F., Method for isolating tight-binding inhibitors of rat lens aldose reductase, *Exper Eye Res*, 79 (2004), 919-26.

[23] Wang, Z., Ling, B., Zhang, R., Suo, Y., Liu, Y., Yu, Z., Liu, C.J., Docking and molecular dynamics studies toward the binding of new natural phenolic marine

inhibitors and aldose reductase, *J. Mol Grap Model*, 28 (2009), 162-9.
[24] Sato, S., Secchi, E.F., Sakurai, S., Ohta, N., Fukase, S., Lizak, M.J., NADPH-dependent reductases and polyol formation in human leukemia cell lines, *Chemico-Biol Interac*, 143 (2003), 363-71.
[25] Miyamoto, S., Molecular modeling and structure-based drug discovery studies of aldose, *Chem-Bio Inform*, 2 (2002), 74-85.
[26] Suzen, S., Recent developments of melatonin related antioxidant compounds, *Com Chem High T Synt*, 9 (2006), 409-19.
[27] Suzen, S., Bozkaya, P., Coban, T., Nebioglu, D., Recent developments of melatonin related antioxidant compounds, *J Enzyme Inh Med Chem*, 21 (2006), 405-11.
[28] Suzen, S., In *Topics in Heterocyclic Chemistry*, Spinger-Verlag, Berlin Heidelberg, 2007
[29] Kikkawa, R., Hatanaka, I., Yasuda, H., Kobayashi, N., Shigeta, Y., Terashima, H., Morimura, T., Tsuboshima, M., Effect of a new aldose reductase inhibitor, (E)-3-carboxymethyl-5-[(2E)-methyl-3-phenylpropenylidene] rhodanine (ONO-2235) on peripheral nevre disorders in streptozotocin-diabetic rats, *Diabetologia*, 24 (1983), 290-2.
[30] Sestanj, K., Bellini, F., Fung, S., Abraham, N., Treasurywala, A., Humber, L., Simard-Dequesne, N., Dvornik, D., N-[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl] thioxomethyl]-N-methylglycine (Tolrestat), a potent, orally active aldose reductase inhibitor, *J Med Chem*, 27 (1984), 255-6.
[31] Ao, S., Shingu, Y., Kikuchi, C., Takano, Y., Nomura, K., Fujiwara, T., Ohkubo, Y., Notsu, Y., Yamaguchi, I., Characterization of a novel aldose reductase inhibitor, FR74366, and its effects on diabetic cataract and neuropathy in the rat, *Metabolism*, 40 (1) (1991), 77-87.
[32] Mylari, B.L., Larson, E.R., Beyer, T.A., Zembrowski, W.J., Aldinger, C.E., Dee, M.F., Siegel, T.W., Singleton, D.H., Novel, potent aldose reductase inhibitors: 3,4-dihydro-4-oxo-3-[[5-m (trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (zopolrestat) and congeners, *J Med Chem*, 34 (1991), 108-22.
[33] Stribling, D., Mirrlees, D.J., Harrison, H.E., Earl, D.C.N., Properties of ICI 128,436, a novel aldose reductase inhibitor, and its effects on diabetic complications in the rat, *Metabolism*, 34 (1985), 336-44.
[34] Chen, X., Zhu, C., Guo, F., Qiu, X., Yang, Y., Zhang, S., He, M., Parveen, S., Jing, C., Li, Y., Ma, B.. Acetic acid derivatives of 3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide as a novel class of potent aldose reductase inhibitors, *J Med Chem*, 53 (2010), 8330-8344.
[35] Chen, X., Yang, Y., Ma, B., Zhang, S., He, M., Gui, D., Hussain, S., Jing, C., Zhu, C., Yu, Q., Liu, Y., Design and synthesis of potent and selective aldose reductase inhibitors based on pyridylthiadiazine scaffold, *Eur J Med Chem*, 46 (2011), 1536- 44.
[36] Yang, Y., Zhang, S., Wu, B., Ma, M., Chen, X., Qin, X., He, M., Hussain, S., Jing, C., Ma, B., Zhu, C., An efficient synthesis of quinoxalinone derivatives as potent inhibitors of aldose reductase, *Chem Med Chem*, 7 (2012), 823-35.
[37] Hamada, Y., Nakamura, J., Clinical potential of aldose reductase inhibitors in diabetic neuropathy, *Treat Endocrinol*, 3 (2004), 245-55.

[38] Matsumoto, T., Yoshiyuki, K.A., Toyosawa, K., Ueda, Y., Bril, V.J., Long-term treatment with ranirestat (AS-3201), a potent aldose reductase inhibitor, suppresses diabetic neuropathy and cataract formation in rats, *Pharmacol Sci*, 107 (2008), 340-8.
[39] Daş-Evcimen, N., Yildirim, O., Suzen, S.. Relationship between aldose reductase and superoxide dismutase inhibition capacities of indole-based analogs of melatonin derivatives, *Arch Biol Scien*, 61 (2009), 675-81.
[40] Ates-Alagoz, Z., Coban, T., Suzen, S., A comparative study: Evaluation of antioxidant activity of melatonin and some indole derivatives, *Med Chem Res*, 1 (2005), 69-179.
[41] Shirinzadeh, H., Ince, E., Westwell, A.D., Gurer-Orhan, H., Suzen, S., Novel indole-based melatonin analogues substituted with triazole, thiadiazole and carbothioamides: studies on their antioxidant, chemopreventive and cytotoxic activities, *J Enzy Inhib Med Chem*, 31(6) (2016), 1312-21.
[42] Gurkok, G., Coban, T., Suzen, S., Melatonin analogue new indole hydrazide/hydrazone derivatives with antioxidant behavior: synthesis and structure-activity relationships, *J Enzy Inhib Med Chem*, 24 (2009), 506-15.
[43] Cerelli, K.J., Curtis, D.L., Dunn, J.P., Nelson, P.H., Peak, T.M., Waterbury, L.D.J., Anti inflammatory and aldose reductase inhibitory activity of some tricyclic aryl acetic acids, *Med Chem*, 29 (1986), 2347-51.
[44] Bradford, M., A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem*, 72 (1976), 248–54.
[45] Suzen, S., Das-Evcimen, N., Varol, P., Sarikaya, M., Preliminary evaluation of rat kidney aldose reductase inhibitory activity of 2-phenylindole derivatives: Affiliation to antioxidant activity, *Med Chem Res*, 16 (2007), 112-18.