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Substituted derivatives of indole acetic acid as aldose reductase inhibitors with antioxidant activity: structure-activity relationship

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Abstract. Although multiple biochemical pathways are likely to be responsible for the pathogenesis of diabetic complications, substantial evidence suggests a key role for the polyol pathway and oxidative stress initiated by hyperglycemia. Thus aldose reductase, the first enzyme of the polyol pathway, has been identified as a potential target of pharmacological intervention to prevent diabetic complications. Aldose reductase inhibitors endowed with antioxidant activity would be dually beneficial. The aim of the study was to evaluate the structure-activity relationship of commercially available indole derivatives supported by the molecular modeling of their interaction with the enzyme aldose reductase from the viewpoint of the inhibitory effect on the enzyme and their antioxidant activity. The partially purified aldose reductase was prepared from rabbit eye lenses. *In vitro* inhibiton of the aldose reductase was determined by a conventional method. Antioxidant action of the compounds was documented in a DPPH test. Marked differences were recorded in the aldose reductase inhibition activities of 1- and 3-indole acetic acid derivatives. The interaction energies of the inhibitor *vs.* enzyme-$NADP^+$ complexes, calculated by computer aided molecular modeling, were in agreement with the higher inhibitory efficacy of 1-indole acetic acid in contrast with 3-indole acetic acid. The more efficient 1-indole acetic acid was proved to create stronger electrostatic interaction with $NADP^+$. However, the order of the antioxidant activities of the compounds studied was not in agreement with that of the inhibitory efficacies.

Key words: Indole acetic acid — Aldose reductase inhibition — Antioxidant — Diabetic complications

Introduction

Links between chronic hyperglycemia and the development of long-term diabetes-specific complications have been proved unequivocally (Brownlee 2005). Clinical studies have demonstrated that close control of blood glucose is significantly effective in reducing diabetic complications, but even optimal control of blood glucose can not prevent the onset of diabetes-related disorders in patients with long-standing disease, suggesting that alternative strategies are needed (Obrosova 2009). Among them, inhibition of aldose reductase (ALR2), the first enzyme of the polyol pathway, is considered a useful therapeutic approach to prevent the onset, or at least to delay the progression and the severity of diabetic complications (Alexiou et al. 2009).

Besides enhancing the flow rate of the polyol pathway, hyperglycemia may contribute to the development of pathological long-term complications by induction of oxidative stress (Baynes and Thorpe 1999). One of the key physiological roles of aldose reductase is the detoxification of toxic aldehydes, lipid peroxidation products, which arise under pathological conditions of oxidative stress. Thus in diabetic conditions, aldose reductase inhibition may have unwanted consequences. The side effect may even be enhanced due to a lack of selectivity for the closely related aldehyde reductase (ALR1), which shares its detoxification role with aldose reductase. In addition, ALR2 itself can be oxidatively modified to enzyme forms that are less sensitive to aldose reductase inhibitors (ARIs), which may affect their responsiveness to ALR2 inhibition therapy (Das and Srivastava 1985; Srivastava et al. 1985; Srivastava et al. 1986a,b; Chandra et al. 2002).

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Thus the effects of ARIs and antioxidants are unidirectional, i.e. both classes of agents interfere with molecular mechanisms involved in the etiology of diabetic complications. Parallel administration of antioxidants and ARIs can counterbalance inhibition of the detoxification role of ALR2. It seems clear that in the case of diseases of multifactorial origin, modulating a single target, even with a very efficient drug, is unlikely to yield the desired outcome. Innovative strategies are focused on rational design of chemical entities able to affect simultaneously multiple key mechanisms. This approach increases the chance of successful therapeutic intervention, decreases the risk of side effects and is economical. ARIs possessing antioxidant activity would therefore seem to be desirable. Compounds such as pyridazines (Coudert et al. 1994), benzopyranes (Costantino et al. 1999) and pyridopyrimidines (La Motta et al. 2007) have been synthesized and display antioxidant as well as aldose reductase inhibitory activities under *in vitro* conditions.

Recently novel carboxymethylated pyridoindoles, analogs of the efficient chain-breaking antioxidant stobadine, have been designed, synthesized and characterized as bifunctional compounds with joint antioxidant/aldose reductase inhibitory activities, with the potential of preventing diabetic complications (Stefek et al. 2008; Juskova et al. 2009, 2010). Other authors recorded the ALR2 inhibition activity of acidic derivatives of structurally related indoles (Da Settimo et al. 2003; Nicolaou and Demopoulos 2003; Sun et al. 2003; Suzen and Buyukbingol 2003; Suzen et al. 2007). The antioxidant activity of indole-based compounds has recently been thoroughly reviewed (Suzen 2006, 2007; Stolc et al. 2006; Suzen et al. 2006; Reiter et al. 2008; Augustyniak et al. 2010; Juranek et al. 2010; Shirinzadeh et al. 2010).

In the light of the above mentioned findings, we considered it interesting to analyze, both experimentally and theoretically by computer aided molecular modeling, the structural aspects of the ALR2 inhibition efficacy and antioxidant activity of 1- and 3-indole acetic acid derivatives shown in Figure l. Considering the ubiquity of indoles in a wide range of natural products, we believe that the common indole core may represent a natural product scaffold as a starting point for "biologically oriented synthesis" (Wilk et al. 2010) of novel biology active agents, as indicated above.

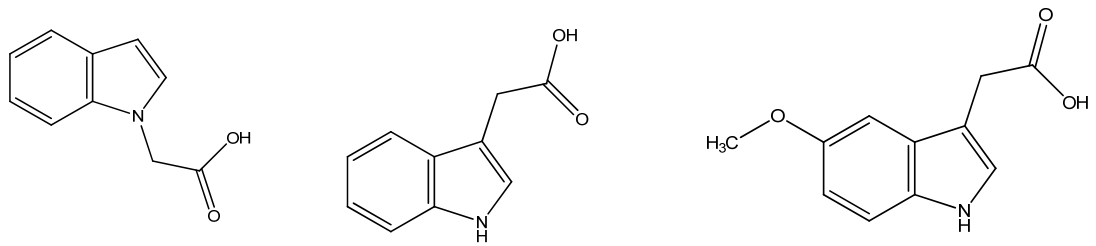
Materials and Methods

Animals

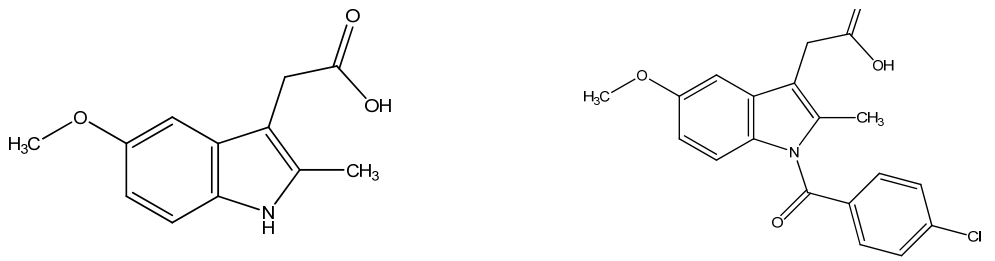
Rabbits HIL (3.5 month old), were used as organ donors. The animals came from the Breeding Facility of the Institute of Experimental Pharmacology and Toxicology Dobra Voda (Slovak Republic). The study was approved by the Ethics Committee of the Institute and performed in accordance with the Principles of Laboratory Animal Care (NIH publication 83-25, revised 1985) and the Slovak law regulating animal experiments (Decree 289, Part 139, July 9th 2003).

Preparation of ALR2

ALR2 from rabbit lens was partially purified using a procedure adapted from Hayman and Kinoshita (1965) as fol-



1-indole acetic acid 3-indole acetic acid 5-methoxy-3-indole acetic acid



5-methoxy-2-methyl-3-indole acetic acid indomethacin

Figure 1. Structures of the commercially available indoles included in the study.

lows: lenses were quickly removed from rabbits following euthanasia and homogenized in a glass homogenizer with a teflon pestle in 5 volumes of cold distilled water. The homogenate was centrifuged at 10 000 × *g* at 0–4°C for 20 min. The supernatant was precipitated with saturated ammonium sulfate at 40%, 50% and then at 75% salt saturation. The supernatant was retained after the first two precipitations. The pellet from the last step, possessing ALR2 activity, was dispersed in 75% ammonium sulfate and stored in smaller aliquots in liquid nitrogen container.

Enzyme assays

ALR2 activities were assayed spectrophotometrically by determining NADPH consumption at 340 nm and were expressed as decrease of the optical density (O.D.)/s/mg protein (Da Settimo et al. 2005). The reaction mixture contained 4.67 mmol/l D,L-glyceraldehyde as a substrate, 0.11 mmol/l NADPH, 0.067 mol/l phosphate buffer, pH 6.2 and 0.05 ml of the enzyme preparation in a total volume of 1.5 ml. The reference blank contained all the above reagents except the substrate D,L-glyceraldehyde to correct for oxidation of NADPH not associated with reduction of the substrate. The enzyme reaction was initiated by addition of D,L-glyceraldehyde and was monitored for 4 min after an initial period of 1 min at 30°C. Enzyme activities were adjusted by diluting the enzyme preparations with distilled water so that 0.05 ml of the preparation gave an average reaction rate for the control sample of 0.020 ± 0.005 absorbance units/min. The effect of inhibitors on the enzyme activity was determined by including in the reaction mixture each inhibitor at required concentrations. The inhibitor at the same concentration was included in the reference blank. Stock solutions of the inhibitors (3 mmol/l) were prepared in water by adding the equivalent amount of NaOH to facilitate dissolution. In the case of 1-indole acetic acid, the IC_{50} value could be determined from the least-square analysis of the linear portion of the semilogarithmic inhibition curves. Each curve was generated using at least four concentrations of inhibitor causing an inhibition in the range from at least 25 to 75%.

DPPH test

To investigate the antiradical activity of the indole acetic acid derivatives, the ethanolic solution of DPPH (50 μmol/l) was incubated in the presence of a compound tested (200 μmol/l) at laboratory temperature. The absorbance decrease, recorded at λ_{max} = 518 nm, during the first 30-min interval was taken as a marker of the antiradical activity.

Computational methods

The geometry of optimal conformers was obtained by conformational analysis by MMFF94 method and consequential optimization by DFT B3LYP/631-G* method in the program Spartan08 (Wavefunction, Inc., Irvine, CA, 2009). The geometry of the complex lidorestat-ALR2-NADP+ (1z3n) from the Protein Data Bank[2] was chosen for molecular modelling. The docking of inhibitor was realized by program AutoDock 4.2. (Morris et al. 2009) and final optimization of enzyme-coenzyme-inhibitor complex was performed in the program VEGAZZ (Pedretti et al. 2004), from which also values of individual interaction energies (calculated by the force field CVFF) were obtained. Dielectric constant was set to value 2 (taking into account the interior of protein) and the original structure of 1z3n was also relaxed till the energy tolerance was fulfilled (0.1 for conjugated gradient minimization). Visualization of the active site with inhibitor was realized by program Spartan08.

Chemicals and instruments

1-Indole acetic acid, 3-indole acetic acid, 5-methoxy-3-indole acetic acid, 5-methoxy-2-methyl-3-indole acetic acid, indomethacin and melatonin were obtained from Sigma-Aldrich (Steinheim, Germany). α,α`-Diphenyl-β-picrylhydrazyl (DPPH) radical, NADPH, D,L-glyceraldehyde were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were purchased from local commercial sources and were of analytical grade quality.

Results

As summarized in Table 1, the most efficient inhibitor of ALR2 is 1-indole acetic acid with IC_{50} ~ 7 μmol/l. Structural variations of 3-indole acetic acid, e.g. 5-methoxy-3-indole acetic acid, indomethacin and its metabolite 5-methoxy-2-methyl-3-indole acetic acid, did not result in significant changes in inhibition efficacy, characterized by the values of IC_{50} > 100 μmol/l.

The interaction energies of 1- and 3-indole acetic acid *vs.* enzyme-$NADP^+$ complexes in comparison with reference

Table 1. Inhibition of rabbit lens aldose reductase

Compound	Inh (%)
1-indole acetic acid	99.26 ± 5.90
3-indole acetic acid	38.36 ± 3.94
5-methoxy-2-methyl-3-indole acetic acid	35.59 ± 12.47
indomethacin	32.78 ± 6.23
5-methoxy-3-indole acetic acid	11.48 ± 6.58

Inh (%), percentage of ALR2 inhibition at inhibitor concentration of 100 μmol/l. IC_{50} for 1-indole acetic acid was 6.96 ± 0.94 μmol/l. Results are means ± SD ($n \geq 3$).

Table 2. Total and individual interaction energies between 1- and 3-indole acetic acids and ALR2

		lidorestat	1-indole acetic acid	3-indole acetic acid
E_{int} total		−102.4	−70.8	−60.2
	total	−30.0	−32.9	−28.7
$NADP^+$	R6R12	8.0	17.9	11.7
	elst	−38.0	−50.7	−40.4
	total	0.8	−0.4	0.3
Tyr48	R6R12	−2.5	−3.3	−2.9
	elst	3.3	2.9	3.2
	total	−4.0	−3.8	−3.4
His110	R6R12	−1.8	−1.6	−1.3
	elst	−2.2	−2.2	−2.1
	total	−12.8	−2.9	−2.9
Trp111	R6R12	−12.5	−3.1	−3.1
	elst	−0.3	0.2	0.2

Individual interaction energies are divided into non–electrostatic (R6R12) and electrostatic (elst) contributions.

lidorestat, calculated by computer aided molecular modeling are summarized in Table 2.

Antioxidant activity of the indole derivatives was studied by a DPPH test. In Table 3 the compounds studied are arranged according to decreasing ability to eliminate the stable free radical of DPPH.

Discussion

Aldose reductase inhibition

Compared to our finding for indomethacin inhibition of rabbit ALR2 (32.8% inhibition at 100 μmol/l), Chaudhry et al. 1983 reported one order higher efficacy (34% inhibition at 10 μmol/l concentration) in relation to human ALR2 isolated from eye lenses. The ALR2 inhibition activities of structurally related derivatives were recorded, namely those of 1-indole butanoic acid with IC_{50} values ranging from 7.4 to 37 μmol/l (Sun et al. 2003), cyano(2-oxo-2,3-dihydroindole-3-ylidene) acetic acid with IC_{50} varying from 0.075 to 5.40 μmol/l (Da Settimo et al. 2003) and (3-benzoylindol-1-yl)acetic acid with 38% ALR2 inhibition at 1 μmol/l concentration (Nicolaou and Demopoulos 2003). Mild ALR2 inhibitory activity of 2-phenylindole derivatives was recorded by Suzen et al. (2007). In the light of the above mentioned results, it appeared interesting to analyze the structural aspects of the marked difference in the inhibition activities of 1- and 3-indole acetic acids, since the common indole core may represent a natural product scaffold as a starting point for biologically oriented synthesis of efficient ARIs.

Lidorestat (Figure 2) and its substitution congeners, derivatives of 1-indole acetic acid (Van Zandt et al. 2005, 2006), belong among the most efficient inhibitors of ALR2 with IC_{50} values in the nmol/l region and with very good ALR2/ALR1 selectivity values. We used the available structure of lidorestat-ALR2 complex (pdb structure 1z3n) for comparative molecular modeling studies of 1- *vs.* 3-indole acetic acids. As reported by Van Zandt et al. (2005), lidorestat was found to interfere with ALR2 *via* two important structural components: i) carboxymethylated indole, which binds to His^{110}, Trp^{111}, Tyr^{48} and $NADP^+$, the principal residues of the active site, ii) substituted benzothiazole, which stabilizes the binding of lidorestat through its π-stacking interaction with the indole moiety of Trp^{111} and hydrogen bonding between the amide N-H of Leu^{300} and the benzothiazole N and 4'fluorine. According to our calculations (Table 2), the former component ensures the main part of the binding, which is mainly of electrostatic nature due to $–COO^–$ and $NADP^+$ interaction. On the other hand, as expected, the calculated interaction energies, presented in Table 2, are in accord with the notion

Table 3. Antiradical activity of the indole derivatives in a DPPH test

Compound	Absorbance decrease (-ΔA/30 min)
5-methoxy-2-methyl-3-indole acetic acid	0.1075 ± 0.012
5-methoxy-3-indole acetic acid	0.0885 ± 0.014
3-indole acetic acid	0.0688 ± 0.014
indomethacin	0.0467 ± 0.017
melatonin	0.0220 ± 0.006
1-indole acetic acid	0.0007 ± 0.008

Ethanolic solution of DPPH radical (50 μmol/l) was incubated in the presence of the compounds tested (200 μmol/l). Absorbance decrease at 518 nm during the initial 30-min period was determined. Results are means ± SD ($n \geq 3$).

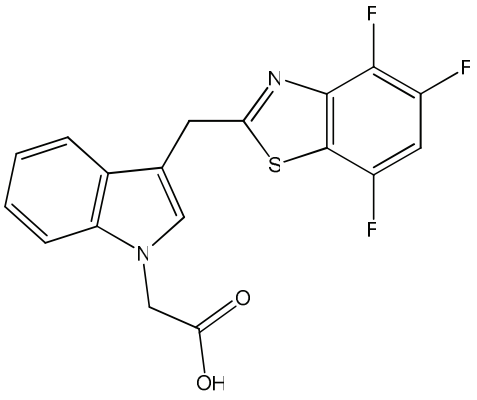


Figure 2. Structure of lidorestat.

that the interaction of the benzothiazole moiety with Trp^{111} is mainly non-electrostatic.

In contrast to lidorestat, both molecules under study, 1-indoleacetic acid and 3-indole acetic acid, lack additional structural moiety able to stabilize total interaction with the ALR2-NADP$^+$ complex. However, as seen in Table 2, their interaction energies (E_{int}) with NADP$^+$, which create the fundamental part of total interaction energy, remain almost the same as for lidorestat itself. The larger value for 1-indole acetic acid (–32.9 kcal/mol) in comparison with 3-indole acetic acid (–28.7 kcal/mol) can be related also to the different dipole values for the original optimized structures (1.8 D for former and 0.8 D for latter).

Based on the distances of the potential partners of the crucial hydrogen bonds between the carboxylic group of the inhibitor and the components of the enzyme binding site, both 1- and 3-indole acetic acid are supposed to create H-bonds both with Tyr^{48} and His^{110}, the residues of the anionic inhibitor binding site of the enzyme (Varnai and Warshel 2000). In addition, 1-indole acetic acid is in a more favorable range of the electrostatic interaction with NADP$^+$ (Figure 3).

The results of molecular modeling are in agreement with experimentally determined higher inhibition efficacy of 1-indole acetic acid in comparison with 3-indole acetic acid.

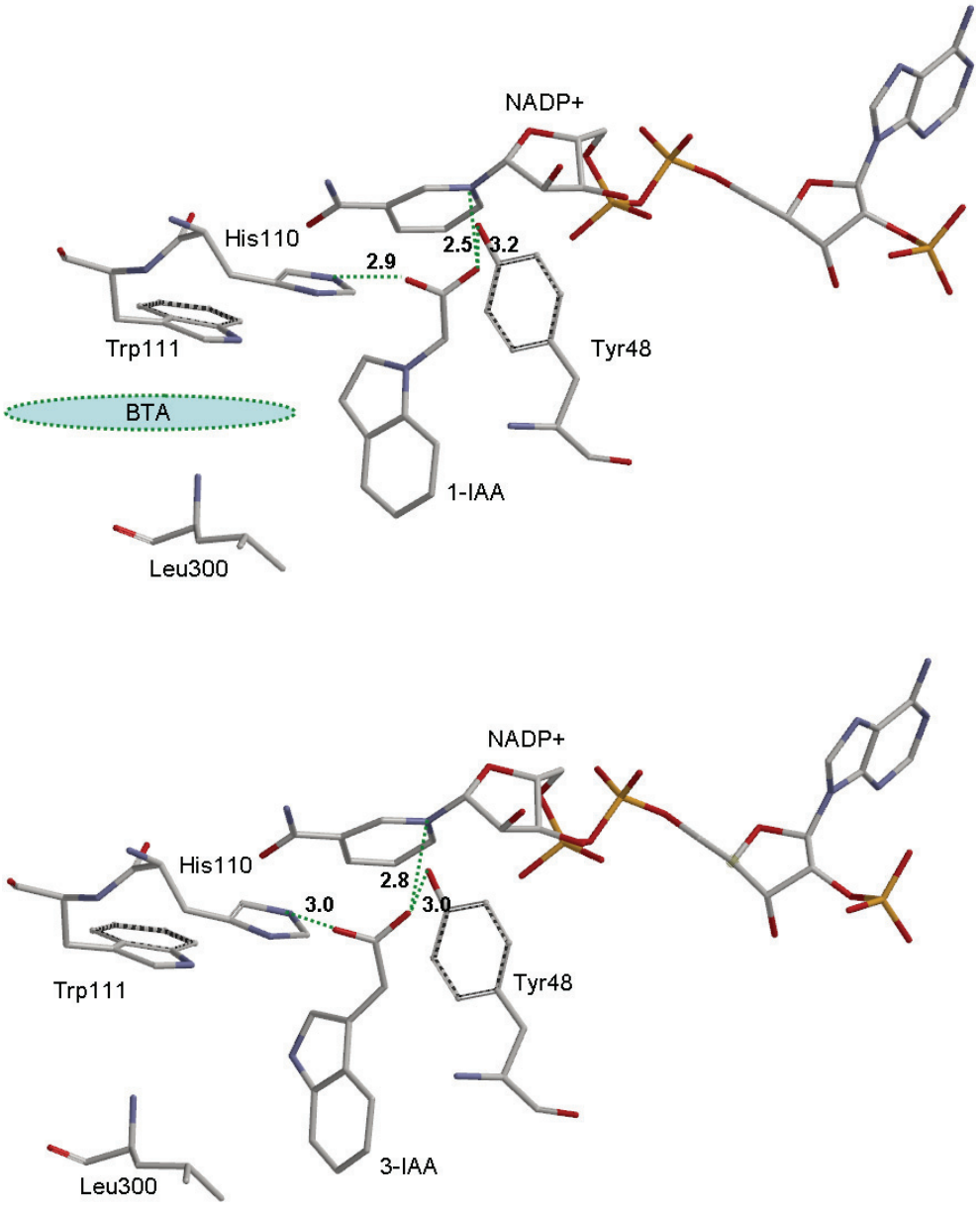


Figure 3. Geometry of the active site for 1-indole acetic acid (up) and 3-indole acetic acid (down) optimized with the whole enzyme-NADP$^+$ complex. Dashed line denotes the hydrogen bonds between the carboxylic oxygens and the hydrogens of His^{110}, Tyr^{48} and the positively charged nitrogen of NADP$^+$. The ellipse on the left side points to the place where benzothiazole (BTA) ring of lidorestat creates significant additive stabilizing interactions with Trp^{111} and Leu 300^{31}.

Antioxidant activity

DPPH, as a weak hydrogen atom abstractor, is considered a good kinetic model for peroxyl ROO· (Blois 1958). All compounds studied, with exception of 1-indole acetic acid, were found to have higher antiradical activity in comparison with melatonin, a 3-substituted indole derivative used as a structurally related reference antioxidant. The absence of the scavenging ability of 1-indole acetic acid is in compliance with the notion according to which the prerequisite of the antiradical activity of the indole derivatives is the presence of free hydrogen at this nitrogen (Rackova et al. 2002). By its splitting, resonance stabilized indolyl radical is created, the stability of which is affected by the presence of aromatic substituents. Electron donor substituents in the positions *orto* and *para* facilitate delocalization of the unpaired electron of the indolyl radical and increase its stability (Rackova et al. 2006). In the case of 1-indole acetic acid, an optional pathway proposed for the interaction of N-substituted indoles with radicals proceeding via electron donation (Reiter et al. 1997; Andreadou et al. 2003) is not obviously valid.

In the group of the 3-indole acetic acid derivatives increased activity of the methoxy derivatives was observed (5-methoxy-2-methyl-3-indole acetic acid and 5-methoxy-3-indole acetic acid) in comparison with 3-indole acetic acid alone. Antioxidant activity of indomethacin, well documented by other authors (Prasad and Laxdal 1994; Kataoka et al. 1997; Stetinova et al. 2002; Fernandes et al. 2004; Costa et al. 2005), results from the conjugation of the aryl substituent with the indole skeleton (Fernandes et al. 2004).

Conclusions

Marked differences were recorded in aldose reductase inhibition activities of 1- and 3-indole acetic acid derivatives. The interaction energies of the inhibitor *vs.* enzyme-$NADP^+$ complex, calculated by computer aided molecular modeling, were in agreement with the higher inhibitory efficacy of 1-indole acetic acid in contrast with 3-indole acetic acid. The more efficient 1-indole acetic acid was proved to create stronger electrostatic interaction with $NADP^+$. However, the order of the antioxidant activities of the compounds studied was not in agreement with that of the inhibitory efficacies.

The results have envisaged design of two lines of potentially biologically active compounds, with relevance (*via* the common indole core) to nature, based on: i) 3-indole acetic acid as a lead for design and optimization of multitarget-oriented compounds with antioxidant efficacy surpassing that of melatonin, yet with mild ARI activity; ii) 1-indole acetic acid as a lead structure, optimization of which may result in highly efficient ARIs, yet devoid of antioxidant action.

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