Secondary metabolites of *Cynodon dactylon* as an antagonist to angiotensin II type 1 receptor: Novel *in silico* drug targeting approach for diabetic retinopathy

R. K. Jananie, V. Priya, K. Vijayalakshmi

Department of Biochemistry, Bharathi Women’s College, Chennai, Tamil Nadu, India

ABSTRACT

**Objectives:** To study the ability of the secondary metabolites of *Cynodon dactylon* to serve as an antagonist to angiotensin II type 1 receptor (AT₁); activation of this receptor plays a vital role in diabetic retinopathy (DR).

**Materials and Methods:** *In silico* methods are mainly harnessed to reduce time, cost and risk associated with drug discovery. Twenty-four compounds were identified as the secondary metabolites of hydroalcoholic extract of *C. dactylon* using the GCMS technique. These were considered as the ligands or inhibitors that would serve as an antagonist to the AT₁. The ACD/Chemsketch tool was used to generate 3D structures of the ligands. A molecular file format converter tool was used to convert the generated data to the PDB format (Protein Data Bank) and was used for docking studies. The AT₁ structure was retrieved from the SwissProt data base and PDB and visualized using the Rasmol tool. Domain analysis was carried from the Pfam data base; following this, the active site of the target protein was identified using a Q-site finder tool. The ability of the ligands to bind with the active site of AT₁ was studied using the Autodocking tool. The docking results were analyzed using the WebLab viewer tool. **Results:** Sixteen ligands showed effective binding with the target protein; diazoprogesteron, didodecyl phthalate, and 9,12-octadecadienoyl chloride (z,z) may be considered as compounds that could be used to bind with the active site sequence of AT₁. **Conclusions:** The present study shows that the metabolites of *C. dactylon* could serve as a natural antagonist to AT₁, that could be used to treat diabetic retinopathy.

**Key words:** Angiotensin II type 1 receptor, *Cynodon dactylon*, diabetic retinopathy, *in silico* analysis

INTRODUCTION

Diabetic retinopathy is a common and progressive microangiopathic complication of diabetes mellitus and a major cause of vision loss and blindness in working age adults worldwide. The predominant cause of vision loss in diabetic patients results primarily from intraocular angiogenesis (proliferative diabetic retinopathy – PDR) and leakage of retinal vessels (diabetic macular edema – DME). These ocular lesions develop and progress despite advances in laser photocoagulation, vitrectomy, and other accessible medical approaches including intensified glycemic control.[1]

The renin–angiotensin system (RAS) is primarily involved in blood pressure regulation and fluid homeostasis. But recently it has been recognized as more than a circulating hormone system in some organs like pancreas, adipose, skeletal and
liver[2] which have their own RAS with distinct functions. RAS is also a causative factor in diabetic microvascular complications including vasoconstriction, inflammation, oxidative stress, cell hypertrophy, proliferation, angiogenesis, and fibrosis.[3] Angiotensin II (Ang II) is a critical active peptide of RAS formed from angiotensin I in a reaction catalyzed by the angiotensin converting enzyme (ACE). Ang II is an antagonist to angiotensin II type 1 receptor (AT1); binding of Ang II to AT1, leads to organ damage via the activation of the vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM)-1, transforming growth factor (TGF-B1), plasminogen activator inhibitor 1 (PAI-1), fibronecton, production of reactive oxygen species (ROS), leading to matrix accumulation, fibrosis, vascular dysfunction, and organ failure.[4]

ACE inhibitors result in tight control of blood pressure and significant suppression and progression of DR but also have the potential risk of hypotension in a large number of normotensive patients. It is therefore considered to be an alternative and novel therapeutic target to control AT1, using AT1 receptor blockers (ARBs). Extensive clinical studies have demonstrated that an AT1 receptor blockade has reduced the occurrence of type 2 diabetes in “high risk patients” by 25%, where preservation of β cell function or improvement of insulin sensitivity is suggested to be the protective mechanism. This finding prompted the investigation into a possible novel role of Ang II in type 2 diabetes and brought new insights into clinical implications. The major components of RAS and angiotensin II type 1 receptor (AT1) have been identified in the retina of human and in ocular tissues of rodents.[5] Activation of AT1, expressed on retinal endothelial cells and pericytes has been implicated in contributing to the microvascular abnormalities in diabetic retinopathy.[6] It has been demonstrated that the administration of ARB inhibited diabetes-induced retinal expression of intercellular adhesion molecule-1 (ICAM)-1 and VEGF and also the cellular and molecular inflammatory parameters in the diabetic retina. These data significantly reveal the contribution of AT1, in diabetic retinopathy, providing a mechanistic reason for targeting AT1 in the treatment of diabetic retinopathy.[6]

Since ancient times plants and plant extracts have been used to combat diabetes. Cynodon dactylon (L.) Pers. (family – Poaceae), which is commonly known as Bermuda grass or Durva in Hindi is traditionally used for diabetes.[7] The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is used in the treatment of catarhral ophthalmia, hysteria, epilepsy, insanity, chronic diarrhea, and dysentery. The plant is a folk remedy for anasarea, calcus, carbuncles, cough, hypertension, snake bites, gout, and rheumatic affections.[8] The secondary metabolite of this plant was identified by GCMS and 24 compounds were identified using the data base of National Institute Standard and Technique (NIST).[9]

The emergence of bioinformatics has provided a platform to explore diseases at their molecular level using computational techniques. In the present study an in silico approach has been carried out to study the inhibitory effect of the secondary metabolites of C. dactylon on AT1, receptor and to study the affinity of these ligands using various bioinformatics tools.

**MATERIALS AND METHODS**

**Preparation of ligands**

The hydroalcoholic extract of C. dactylon was analyzed using the GCMS technique and the compounds in it were identified using NIST. These secondary metabolites were used as ligands for this study. The two-dimensional structures of these ligands were generated using the ACD/ChemSketch tool. This software contains tools for 2D cleaning, 3D optimization, and viewing. These data are saved as a molecular format file (MDL MOL format). The molecular format converter tool (http://www.webqc.org/molecularformatsconverter.php) is used to convert this file into the Protein Data Bank format and is used during docking analysis.

**Retrieval of target protein sequence**

The protein sequence for the angiotensin II type 1 receptor (AT1) was obtained from the protein sequence data base of Uniprot (http://www.uniprot.org/uniprot/p30556). It was ascertained that the three-dimensional structure of AT1 was available in the PDB data base. The structure was visualized using the Rasmol Tool.

**Domain analysis**

Pfam is a large collection of multiple sequence alignments and hidden Markov models covering many protein domains. Pfam deals with multiple domain proteins. Pfam families match 75% of protein sequences in Swiss-Prot and TrEMBL (and 53% of all residues). The functional analysis of AT1 was predicted using the Pfam data base (http://www.pfam.sanger.ac.uk/)

**Active site prediction**

After obtaining the 3D structure, the possible binding sites of AT1 were searched using Q-site finder (http://www.modelling.leeds.ac.uk/qsitefinder/). Ten binding sites were obtained of which the first site is usually taken as the active site for docking analysis since it is the most conserved region.

**Docking the inhibitors with the active site of the angiotensin II type 1 receptor (AT1)**

Totally 24 ligands were docked with AT1 using the Lamarckin genitic algorithm (LGA) provided by the AutoDock Program, Version 3.0 (http://www.autodock.scripps.edu/). Polar hydrogen was added to the receptor and Kollaman charges were assigned and salvation parameters were added with “Addsol” option in AutoDock. For the inhibitors, charges of the Gasteiger type were assigned. The internal degree of freedom and torsion were defined using the “Ligand Torsions” menu option of AutoDock. The grid maps representing the protein were calculated using the “AutoGrid” option. The protein was
centered on the geometric center prior to docking. Docking stimulations were carried out with an initial population of 50 individuals and a maximum of 25,000 energy evaluations were used as the docking parameters for obtaining the final docking structures. In addition to returning the docked structure, AutoDock also calculates an affinity contrast for each ligand-receptor configuration. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were analyzed using the WebLab viewer tool.

Lipinski drug filter analysis

The ligands used in the present study were subjected to Lipinski rule screening using the tool Lipinski Drug Filter of the Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbioiitd.res.in/utility/lipinskifilter.jsp) according to which prediction of a high probability of success or failure is based on drug likeness for molecules complying with two or more of the rules namely molecular weight less than 500 Dalton, high lipophilicity (expressed as Log P less than 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and polar surface area between 0.0 and 150 Angstroms.

RESULTS

The structures of the ligands were drawn using the ACD/ChemSketch tool and converted into the PDB format using a molecular converter tool. Table 1 shows the compounds used as ligands for this study. The sequence of the angiotensin II type 1 receptor was retrieved from the Swissprot database (Swissprot ID: P30556). The 3D structure of AT₁ (PDB ID: 1ZVO B chain) was downloaded from the PDB data base and visualized using the Rasmol tool as shown in Figure 1. The functional region of AT₁, predicted from Pfam was identified as 7 transmembrane receptor 7tm_1 (region 45-302), a single domain region, which belongs to the rhodopsin family. The possible active sites of AT₁ were identified using the Q-site finder, which produced 10 binding sites of which site 1, was considered highly conserved, and chosen as the most favorable site for docking analysis. Figure 2 shows the AT₁ protein with the active site and its residues. Out of the 24 ligands which were AutoDocked with the AT₁ receptor, only 16 compounds were able to form hydrogen bonds with the active site of target protein AT₁. The final docked conformation obtained for the different inhibitors was evaluated based on the number of hydrogen bonds formed and their docking scores [Table 2]. Some of the docked configurations (AT₁ complex with the ligands) are shown in Figure 3. The key interacting sites of AT₁ are HD21 of Asn (asparagine) 298; OD2, of Asp (aspartic acid) 74; OD1, ND2, HD22 of Asn (asparagine) 46; O, HD22, ND2 of Asn (asparagine) 295; O of Ile (isoleucine) 242; O of Leu (leucine) 122; HD21 of Asn (asparagine) 298; O of Val (valine) 41; O of Gly (glycine) 42; NE1 of Trp (tryptophan) 253; OD1, O, H of Asn 294; OH of Tyr (tyrosine) 113. The result of Lipinski’s rule suggests the analyzed compounds as the best therapeutic drug [Table 3]. In the present study, 16 compounds were able to form hydrogen bonds with the angiotensin II type 1 receptor and satisfied more than two rules predicting a high probability of success to show drug likeness. Didodecyl phthalate satisfied three rules. Linoleic acid ethyl ester, 9,12-octadecadienoyl chloride(z,z)-, hexadecanoic acid 2-hydroxy-1-[hydroxymethyl]ethyl ester, 13-tetradece-11-yn-1-ol, 9,12-octadecanoic acid satisfied four rules, and rest of the compounds satisfied all the five rules.

DISCUSSION

The results of the present study revealed that 16 ligands displayed a good docking score and drug likeness. In general...
the lead compound is selected based on the least docking score, the number of hydrogen bonds formed with the target protein. That compound should also satisfy the maximum Lipinski rule. Among the 16 compounds linoleic acid ethyl ester had the least docking score of −10.40 kcal/mol but formed only four hydrogen bonds with angiotensin II type 1 receptor and satisfied only four Lipinski rules. Glycerin formed seven hydrogen bonds with AT\(_1\) which was the maximum among the compounds analyzed but its docking score was −4.05 kcal/mol. The docking score of ethyl à-d-glucopyranoside was −7.33 kcal/mol, and it formed six hydrogen bonds with the target protein and also satisfied the entire five Lipinski rules.

According to a survey of literature, pressor doses of Ang II inhibited insulin release and enhanced insulin sensitivity in healthy subjects.[10] It is proved that chronic stimulation of Ang II signaling could act through AT\(_1\) to trigger beta cell apoptosis, inhibition of insulin release, and dysfunction mediated by oxidative stress.[11] Blockade of Ang II signaling by AT\(_1\) receptor blockers (ARB) improved beta cell function and glucose tolerance in a mouse model of type 2 diabetes.

Table 1: Compounds present in the extract of *Cynodon dactylon* identified using GCMS analysis

| Name of the compound | Molecular formula |
|----------------------|------------------|
| Glycerin             | C3H8O3           |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | C6H8O4 |
| Thymol               | C10H14O          |
| Conhydrin            | C8H17NO          |
| 1,2-Cyclopentanediol, 3-methyl- | C6H12O2 |
| Benzenepropanol, 4-hydroxy-a-methyl-, (R)-  | C10H14O2 |
| Ethyl à-d-glucopyranoside | C8H16O6 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | C20H40O |
| n-Hexadecanoic acid | C16H32O2         |
| Hexadecanoic acid, ethyl ester | C18H36O2 |
| Phytol               | C20H40O          |
| Linoleic acid ethyl ester | C20H36O2 |
| 9,12-Octadecadienol chloride, (2,2)- | C18H31ClO |
| Octadecanoic acid, ethyl ester | C20H40O2 |
| Pentanal, 2-methyl-  | C6H12O           |
| 1-(Cyclopropyl-nitro-methyl)-cyclopentanol | C9H15NO3 |
| 2-Propanamide, N-[2-(dimethylamino)ethyl]- | C7H14N2O |
| Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester | C19H38O4 |
| Didodecyl phthalate  | C32H54O4         |
| 13-Tetradec-11-yn-1-ol | C14H24O |
| 10-Undecyn-1-ol      | C11H20O0         |
| Squalene             | C30H60           |
| 9,12-Octadecadienoic acid (2,2)-, phenylmethyl ester | C25H38O2 |
| Diazoprogesterone    | C21H30N4         |

Figure 3: Docked configuration of AT\(_1\) receptor with the ligands and the hydrogen bonds between the active site of AT\(_1\) receptor and the ligands as deciphered using WebLab viewers
Table 2: Docking score and number of hydrogen bonds formed between angiotensin II type 1 receptor with various inhibitors

| Ligand                                                                 | Docking score (kcal/Mol) | No. of hydrogen bonds formed |
|------------------------------------------------------------------------|--------------------------|------------------------------|
| Glycerin                                                              | −4.05                    | 7                            |
| 4H-pyran-4-ome-2, 3-dihydro-3, 5-dihydroxy-6-methyl                   | −6.03                    | 1                            |
| Thymol                                                                | −5.98                    | 1                            |
| Conhydrin                                                             | −5.74                    | 3                            |
| Benzenepropanol, 4-hydroxy-a-methyl-(r)                               | −6.76                    | 3                            |
| Ethyl a-d-gluco pyranoside                                           | −7.33                    | 6                            |
| Linoleic acid ethyl ester                                            | −10.40                   | 4                            |
| 9, 12-Octadecadienoyl chloride, (2,2)                                 | −10.62                   | 1                            |
| 1, (Cyclopropyl-nitro-methyl), cycloptanol                            | −5.89                    | 5                            |
| 2-Propenamide, n-[2-(dimethyl amino) ethyl]                            | −6.07                    | 2                            |
| Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl] ester                  | −9.68                    | 3                            |
| Didodecyl phthalate                                                   | −12.97                   | 1                            |
| 13-tetra dece-11-yn-1-ol                                             | −9.42                    | 2                            |
| 10-Undecyn-1-ol                                                       | −7.95                    | 1                            |
| 9, 12-Octadecadieneic acid (2,2)-, phenylmethyl ester                 | −11.84                   | 1                            |
| Diazoprogesterone                                                     | −9.55                    | 3                            |

Table 3: Results of Lipinski’s drug filter analysis

| Compound name                                                                 | Molecular Wt. (Dalton) | Donors | Acceptors | Polar surface area | Log P |
|-----------------------------------------------------------------------------|------------------------|--------|-----------|--------------------|-------|
| Glycerin                                                                    | 92.0                   | 3      | 3         | 60.69              | −1.88 |
| 4H-pyran-4-ome-2,3-dihydro-3,5-dihydroxy-6-methyl                          | 144.1                  | 2      | 4         | 66.76              | 0.08  |
| Thymol                                                                      | 150.1                  | 1      | 1         | 20.23              | 3.25  |
| Conhydrin                                                                   | 143.1                  | 2      | 2         | 32.26              | 0.83  |
| Benzenepropanol, 4-hydroxy-a-methyl-(r)                                     | 166.1                  | 2      | 2         | 40.46              | 1.78  |
| Ethyl a-d-glucopyranoside                                                   | 208.1                  | 4      | 6         | 99.38              | −1.61 |
| Linoleic acid ethyl ester                                                  | 308.2                  | 0      | 2         | 26.32              | 6.92  |
| 9, 12-Octadecadienoyl chloride, (2,2)-                                     | 298.7                  | 0      | 1         | 17.07              | 6.84  |
| 1-(Cyclopropyl-nitro-methyl)-cyclopentanol                                  | 185.1                  | 1      | 4         | 60.21              | 2.21  |
| 2-Propenamide, N-[2-(dimethyl amino)ethyl]                                  | 142.1                  | 1      | 3         | 32.34              | −0.15 |
| Hexadecanoic acid 2-hydroxy-1-[hydroxymethyl]ethyl ester                   | 330.2                  | 2      | 4         | 66.76              | 5.03  |
| Didodecyl phthalate                                                        | 502.3                  | 0      | 4         | 52.60              | 11.80 |
| 13 Tetradece -11-yn-1-ol                                                    | 208.2                  | 1      | 1         | 20.23              | 5.37  |
| 10-Undecyn-1-ol                                                            | 168.1                  | 1      | 1         | 20.23              | 3.70  |
| 9, 12-Octadecadieneic acid (2,2)-, phenylmethyl ester                      | 370.3                  | 0      | 2         | 26.30              | 8.21  |
| Diazoprogesterone                                                          | 338.3                  | 0      | 4         | 78.8               | 4.26  |

Expression and production of Ang II and AT1 were upregulated in the retina of diabetic induced animal models. Retinal vasculature was suppressed by blocking AT1 signaling. Recent clinical laboratory data revealed the elevated levels of Ang II in the vitreous samples from patients with proliferative diabetic retinopathy and diabetic macular edema. The hydroalcoholic extract of *C. dactylon* was given orally to Wistar rats induced with diabetes for a period of 20 weeks and many biochemical parameters were studied. Histopathological studies showed no changes in the retina in the treated group. While in the untreated diabetes ARB significantly prevents diabetes-induced oxidative damage and also acts as a protective agent against diabetes-induced cellular damage. The compounds present in the hydroalcoholic extract of *C. dactylon* showed significant binding with the angiotensin II type 1 receptor in the present study, suggesting that these secondary metabolites could be used as a natural source of ARBs.
group, retinal edema and neovascularization were observed (unpublished data already sent for publication). These findings reveal that the extract prevented the complications arising out of DR. The high concentration of the target protein in the vitreous of DR patients could be suppressed via the inhibitory effect of compounds present in C. dactylon. Since the molecular weight of these compounds is low, they can easily pass the retinal blood barrier to reach the vitreous. Blocking of Ang II signaling via AT₁, showed a suppressive effect on retinal neovascularization and retinal inflammation. The present data indicate AT₁ blockade as a possible therapeutic strategy for preventing diabetic retinopathy. Valsartan (Val), Candesartan (Cand), Losartan (Lo), and Olmesartan (Olm) are drugs of the sartan family which are currently used as ARBs; their effectiveness in therapy differs. This may be related to their binding strength. Valsartan interacts with the residues of Ser 105, Ser 109 of transmembrane (TM) 3, Lys199 TM 5, and Asn 295 TM 7 with a docking score of −12.24 kcal/mol. Olmesartan interacts with Try113 TM 3 and His 256 TM 6[14] Candesartan with Lys199 TM 5, Asn 295 TM 7, Ser 109 TM 3, and Gln 257 TM 6. Losartan binds with Asn 295 TM 7 and Ser 109 TM 3. Binding sites of these drugs lie between TM 3, 5, 6, and 7 of the AT₁ receptor.[15] Movement of TM3 is known to be responsible for an initial step in the activation of the AT₁ receptor.[16] Binding to TM6 is also essential in AT₁ antagonist studies as in Olmesartan and Candesartan. Losartan and Cadesartan also bind to Ans 295 in TM 7.

In the present study diazoprogesteron binds to Try 113 of TM 3, like Olmesartan and Asn295, Asn 294 of TM 7 as in Candesartan and Losartan with a free energy (Docking score) of −9.55.

Didodecyl phthalate binds to Try 253 of TM 6 as in Candesartan and Olmesartan with a free energy of −12.97. 9, 12-octadecadienoyl chloride (zz) has a free energy of −10.62 and interacts with Leu 112 of TM 3. Valsartan, Candesartan, Losartan, and Olmesartan all these drugs also interact with transmembrane 3. The present in silico analysis revealed that diazoprogesteron, didodecyl phthalate, and 9,12-octadecadienoyl chloride (zz) may be used as natural antagonist to AT₁ receptors. The results are computational analysis of the compounds in the extract which act as inhibitors to AT₁, but however to confirm these results in vivo studies can be carried out to prove the efficacy of these compounds.

CONCLUSION

The interaction of compounds diazoprogesteron, didodecyl phthalate, and 9, 12-octadecadienoyl chloride (zz) with angiotensin II type 1 receptor is similar to that of the commercial drugs used as angiotensin receptor blockers (ARBs) and can also be used to treat diabetic retinopathy. Crude extracts are used in Indian medicine, so that the effect of the drug may be due to the synergistic activity of the compounds present in them. From the present in silico analysis, it could be inferred that the secondary metabolites of C. dactylon are capable of interacting with many active sites of the target protein and may serve as a natural source of ARBs and a natural therapeutic agent in treating diseases and complication including diabetic retinopathy that arise due to the signaling of angiotensin II. However, further studies have to be carried out to isolate these compounds present in the extract which can act as inhibitors to angiotensin II type 1 receptors and studied in diabetes induced retinopathy rat models to confirm the efficacy of C. dactylon extract.

REFERENCES

1. Moss SE, Klein R, Klein BE. The 14-year incidence of visual loss in a diabetic population. Ophthalmology 1998;105:994-1003.
2. Chu KY, Leung PS. Angiotensin II in type 2 diabetes mellitus. Curr Protein Pept Sci 2009;10:75-84.
3. Wilkinson-Berka JL. Angiotensin and Diabetic Retinopathy. Inter J Biochem Cell Biol 2006;38:752-65.
4. Wilkinson-Berka JL, Duncan J, Campbell. (Pro) renin receptor: A treatment target for Diabetic Retinopathy? Diabetes 2009;58:1485-87.
5. Clermont A, Bussell SE, Feener EP Role of the Angiotensin II Type 1 receptor in the pathogenesis of Diabetic Retinopathy: effect of blood pressure control and beyond. J Hypertens Suppl 2006;24:573-80.
6. Nagai N, Izumi-Nagai K, Oike Y, Koto T, Satofuka S, Ozawa Y, et al. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappa B pathway. Invest Ophthalmol Vis Sci 2007;48:4342-50.
7. Kirtikar KK, Basu BD. Indian Medicinal Plants. India: Lalit Mohan Publication; 1980. p. 2650.
8. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi: Publication and Information Directorate; 1999. p. 88.
9. Jananie RK, Priya V, Vijayalakshmi K. Determination of bioactive components of Cynodon dactylon by GCMS analysis. NY Sci 2011;4:16-20.
10. Townsend RR, Di Pette DJ. Pressor doses of Angiotensin II increases insulin-mediated glucose uptake in normotensive men. Am J Physiol 1993;265:E362-66.
11. Lapw R, Del GS, Bugliani M, Boggi U, Mosca F, Torri S, et al. The direct effect of Angiotensin –converting enzyme inhibitors zofenoprilat and enalaprilat, on isolated human pancreatic islets. Eur J Endocrinol 2006;154:355-61.
12. Chu KY, Lau T, Carlsson PO, Leung PS. Angiotensin II type 1 receptor blockade improves β-cell function and glucose tolerance in a mouse model of type 2 diabetes. Diabetes 2006;55:367-74.
13. Ozdemir S, Tandogan B, Ulusu NN, Turan B. Angiotensin II receptor blockade prevents diabetic induced oxidative damage in rat heart. Folia Biol 2009;53:11-6.
14. Miura S, Kiya Y, Kanazawa T, Inaizumi S, Fujino M, Matsuo Y, et al. Differential bonding interactions of inverse agonists of angiotensin II type 1 receptor in stabilising the inactive state. Mol Endocrinol 2008;22:139-46.
15. Bhuiyan MA, Ishiguro M, Hossain M, Nakamura T, Orito M, Miura S, et al. Binding sites of valsartan, candesartan and losartan with angiotensin II receptor 1 subtype by molecular modeling. Life Sci 2009;85:136-40.
16. Miura S, Saku K, Naruki SS. Molecular analysis of the structure and function of the Angiotensin II type 1 receptor. Hypertens Res 2003;26:937-43.