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Research Article

Inhibition profiles and molecular docking studies of antiproliferative agents against aldose reductase enzyme

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ABSTRACT

Inhibition of Aldose Reductase (AR) is very important in terms of preventing many diabetic complications such as retinopathy, neuropathy, and cataract. In this study, inhibition effects of some antiproliferative agents, which have been shown to have many biological activities besides their anticancer properties, on the AR enzyme, which is a diabetes-related enzyme, were investigated. Biochanin A compound with an IC_{50} value of 4.44 µM showed the best inhibition effect. IC_{50} values of Rhein, Betulinic acid, Sanguinarine chloride, Budesonide, Plumbagin and 2-Methoxyestradiol compounds were calculated as 7.87 µM, 7.45 µM, 19.25 µM, 21.00 µM, 28.87 µM and 38.5 µM, respectively. Molecular docking studies have also been conducted to elucidate the inhibition mechanisms of the compounds whose *in vitro* inhibition effects have been investigated, and the free binding energies of enzyme-inhibitor complexes have been calculated with the Molecular Mechanics Generalized Born Surface Area (MM-GBSA). Both experimental data and computer-aided calculations have revealed that the compounds studied are very important drug candidates aimed at preventing diabetic complications.

Keywords: Aldose reductase, antiproliferative, molecular docking, inhibition.

Antiproliferatif ajanların aldoz redüktaz enzimine karşı inhibisyon profilleri ve moleküler docking çalışmaları

ÖZ

Aldoz Redüktaz (AR)'ın inhibisyonu, retinopati, nöropati, katarakt gibi birçok diyabetik komplikasyonun önlenmesi açısından oldukça önemlidir. Bu çalışmada, antikanser özelliklerinin yanı sıra birçok biyolojik aktiviteye sahip oldukları gösterilen bazı antiproliferative ajanların diyabet ilişkili bir enzim olan AR enzimi üzerine inhibisyon etkileri araştırılmıştır. En iyi inhibisyon etkisini IC_{50} değeri 4.44 µM olarak bulunan Biochanin A bileşiği göstermiştir. Rhein, Betulinic acid, Sanguinarine chloride, Budesonide, Plumbagin ve 2-Methoxyestradiol bileşiklerinin IC_{50} değerleri, sırasıyla, 7.87 µM, 7.45 µM, 19.25 µM, 21.00 µM, 28.87 µM ve 38.5 µM olarak hesaplanmıştır. *In vitro* inhibisyon etkileri incelenen bileşiklerin inhibisyon mekanizmalarını aydınlatmak amacıyla moleküler docking çalışmaları da yapılmış ve enzim-inhibitör komplekslerinin, Molecular Mechanics Generalized Born Surface Area (MM-GBSA) ile serbest bağlanma enerjileri hesaplanmıştır. Hem deneysel veriler hem de yapılan bilgisayar destekli hesaplamalar çalışılan bileşiklerin diyabetik komplikasyonların önlenmesini hedefleyen çok önemli birer ilaç adayı olduklarını ortaya koymuştur.

Anahtar Kelimeler: Aldoz redüktaz, antiproliferative, moleküler docking, inhibisyon.

1. INTRODUCTION

Long-term hyperglycemia in diabetes mellitus is regarded to be the primary cause of long-term diabetic disorders such nephropathy, retinopathy, cataractogenesis, and neuropathy. Excessive oxidative stress, increased advanced glycation endproduct (AGE) synthesis, and an augmented aldose reductase-related polyol pathway have all been identified as possible paths to explain the pathophysiology of diabetic problems.[1] Aldose Reductase (AR) is a monomeric reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme that belongs to the aldo-keto reductase superfamily. Aldose reductase's primary function is to convert toxic aldehydes in the cell to inert alcohols, but when glucose levels in the cell become too high, it also transforms glucose to sorbitol, which is subsequently oxidized to fructose.[2]

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AR is found in nearly all mammalian cells, as well as in elevated amounts in certain tissues including the retina, lens, and sciatic nerves, which are quickly compromised by diabetic complications linked to increased polyol pathway flux which can lead to cataract.[3] Furthermore, because of their low penetration through membranes and inadequate metabolism, sorbitol and its metabolites concentrate in the eye, nerves, and kidneys, leading to the generation of diabetic complications such as nephropathy, retinopathy, and neuropathy.[4] The AR enzyme has also been reported to be associated with many other diseases, including inflammation,[5] cardiovascular diseases,[6,7] ovarian abnormalities,[8,9] depression,[10,11] and cancer.[12] In addition, numerous studies have demonstrated that inhibition of AR prevents restenosis, endothelial cell apoptosis, and vascular smooth muscle growth.[13,14]

As a result, AR inhibitors (ARIs) could be a possible therapeutic option for treating and preventing diabetic complications through suppressing the hyperglycemia-induced polyol pathway flux. So, in our presented study, we investigated inhibition effects of some anti-proliferative agents on diabetes mellitus-related aldose reductase enzyme. Molecular docking studies have also been conducted to elucidate the inhibition mechanisms, and Molecular Mechanics Generalized Born Surface Area (MM-GBSA) free binding energies of anti-proliferative agents-AR complexes have been calculated.

2. MATERIALS AND METHODS

2.1. Chemicals

β-nicotinamide adenin dinucleotide phosphate (β-NADPH), DL-glyceraldehyde, Na-phosphate, recombinant human aldose reductase enzyme, and antiproliferative agents were obtained from Sigma-Aldrich Co. (Taufkirchen, Germany).

2.2. The AR activity assay

The AR enzyme activity was measured using a modified version of Cerelli et al (1986)'s protocol.[15] To begin the reaction, a 1 mL reaction medium was made by combining 0.45 mL deionized water, 0.1 mL NADPH, 0.25 mL Na-phosphate buffer, 0.1 mL enzyme solution, and 0.1 mL DL-glyceraldehyde. Spectrophotometric monitoring of the decline in NADPH concentration at 340 nm was used to assess enzyme activity.

2.3. *In Vitro* inhibition studies

The anti-proliferative agents Biochanin A, Rhein, Sanguinarine chloride, 2-Methoxyestradiol, Budesonide, Plumbagin, and Betulinic acid were tested for their ability to inhibit the AR enzyme. Each antiproliferative agent was prepared in five separate concentration, and the inhibitory actions of these compounds on the AR enzyme were investigated. The enzyme activity in the inhibitor-free control cuvette was assumed to be 100%, and inhibitor concentrations that halved the enzyme activity were calculated (IC_{50} values).

2.4. Molecular docking studies

To assess the potential interactions of the studied anti-proliferative agents with the target enzyme, AR, molecular docking simulations were run. To conduct molecular docking simulations, the Maestro 12.5 program, which is part of the Schrödinger Molecular Modeling Suite program, was used.[16] To begin, the RCSB Protein Data Bank (PDB) was used to obtain the X-ray crystal structure of the AR enzyme (PDB ID: 2FZD). The Protein Preparation Wizard was used to pre-process and prepare the enzyme crystal structure under physiological conditions. The binding order and charges were allocated using the protein preparation wizard,[17] all missing hydrogen atoms were inserted, and missing side chains of the protein were filled using the Prime module of the Maestro 12.5. With the assistance of Maestro's Ligprep software, 2D structure sketches of all ligands to be docked, their transformation into 3D structures, their protonation states at pH 7.4, and their optimizations were carried out. After preparing the protein structure and ligands to be docked, anti-proliferative agents were docked against the AR enzyme using Glide/XP (extra precision).[18] The Glide software[19] was used to measure the anti-proliferative agents' binding energies and docking scores to the AR target.

2.5. Calculations of binding free energy via molecular mechanics/generalized born surface area (MM/GBSA)

The Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) approach combines molecular mechanics equations with continuum solvation models to measure binding free energies (ΔG_{bind}) for macromolecules.[20] Prime/MM-GBSA, which uses the OPLS3e force field and the VSGB dissolvable model, is used to calculate the binding free energies of AR-anti-proliferative agent complexes.[21]

3. RESULTS AND DISCUSSION

The polyol pathway, which becomes active when blood sugar levels rise, is regarded to be the leading cause of diabetic disorders. Aldose reductase, the initial stage of the polyol pathway, is related in particular to diabetes sequelae such as retinopathy, neuropathy, cataractogenesis, and nephropathy.[22] Therefore, inhibitors of aldose reductase can be helpful therapeutic drugs for diabetic complication treatment and prevention.[23] New and efficient aldose reductase inhibitors need to be identified in order to better the

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quality of life of diabetes patients by preventing diabetes complications. In this connection, our current work looked at the *in vitro* inhibition impact of certain antiproliferative agents on the activity of the human recombinant aldose reductase enzyme. *In vitro* inhibition results of antiproliferative agents on aldose reductase enzyme are collectively given in Table 1.

Table 1. *In vitro* inhibition results of antiproliferative agents against aldose reductase enyzme

Compound	IC_{50} (µM)	R^2
Biochanin A	4.440	0.926
Rhein	7.870	0.947
Betulinic acid	7.450	0.980
Sanguinarine chloride	19.25	0.957
Budesonide	21.00	0.930
Plumbagin	28.87	0.978
2-Methoxyestradiol	38.50	0.930

In addition, binding modes and binding energies were also calculated by molecular docking simulations to evaluate the inhibition mechanism of antiproliferative agents whose inhibition effects on the recombinant human enzyme were investigated. However, molecular mechanical Generalized Born Surface Area (MM / GBSA) method was used to calculate the free binding energies of AR-antiproliferative agent complexes. The results of molecular docking simulations and MM-GBSA free binding energies are given in Table 2. Sorbinil, which is a standard inhibitor for AR enzyme, was used as a positive control compound.

Table 2. Docking scores, binding energies and Prime MM-GBSA free binding energies (ΔG) of antiproliferative agents for AR enzyme

Name	Docking Score	XP GScore	Glide evdw	Glide emodel	MM-GBSA (ΔG_{bind})
Biochanin A	-10.04	-10.35	-32.73	-57.28	-62.17
Rhein	-9.72	-9.72	-28.44	-53.08	-42.67
Sanguinarine chloride	-9.09	-9.10	-30.43	-51.74	-56.96
2-Methoxyestradiol	-8.82	-8.82	-23.30	-33.57	-49.82
Budesonide	-8.29	-8.29	-24.24	-48.17	-50.92
Plumbagin	-7.35	-7.63	-20.17	-30.91	-38.29
Betulinic acid	-5.83	-5.84	-21.27	-34.80	-26.70
Sorbinil	-7.81	-7.86	-28.89	-43.59	-42.61

Biochanin A compound, which was previously determined to have antioxidant, anti-inflammatory, anticancer, anti-microbial, neuroprotective and hepatoprotective properties,[24] has a very strong inhibition effect (IC_{50}=4.44 µM) against the AR enzyme. Molecular docking studies of the Biochanin A compound against the AR enzyme revealed that the compound constructed hydrogen bonding with the amino acid residues TYR48 and HIS110 and a π-π interaction with TRP111 at the active site of the enzyme (Figure 1). The compound was determined to have better docking score and lower free binding energy (ΔG) than the positive control compound, sorbinil (Table 2).

It has been shown in the literature that Betulinic acid and Rhein compounds, which we found to have close and strong inhibitory effects as a result of our *in vitro* inhibition studies (IC_{50}=7.45 µM and 7.87 µM, respectively), have nephroprotective, hepatoprotective, anti-inflammatory, anticancer, anti – HIV, antioxidant, antimalarial and antimicrobial activities.[25,26] Molecular docking studies for Rhein and Betulinic acid compounds, which show strong experimental AR inhibitory effects, confirmed the inhibition effects of the compounds (docking scores, 9.72 and 5.83, respectively). Rhein compound established three hydrogen bonding with the residues TYR48, HIS110 and TRP111 and dual π-π stackings with the amino acid residue TRP20 in the active site of the AR enzyme (Figure 2). Similar to the Rhein compound, Betulinic acid hydrogen bonded to the Trp20 amino acid residue in the AR active center and also interacted hydrophobically with amino acids such as TYR48 and TRP111 (Figure 3).

IC_{50} values of other antiproliferative agents, Sanguinarine chloride, Budesonide, Plumbagin and 2-Methoxyestradiol compounds, whose inhibition effects against AR enzyme were investigated, were found in the

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range of 19.2-38.5 µM. It can be said that these compounds (Sanguinarine chloride, Budesonide, Plumbagin and 2-Methoxyestradiol), which have a wide range of biological activities, have antidiabetic effects, considering their inhibition effects for the determined AR enzyme. Molecular docking studies for these compounds reveal that all of the compounds scored much better than the positive control compound, sorbinil (Table 2). In addition, in the MM-GBSA study, the compounds (except Plumbagin) were also found to have better free binding energies than sorbinil.

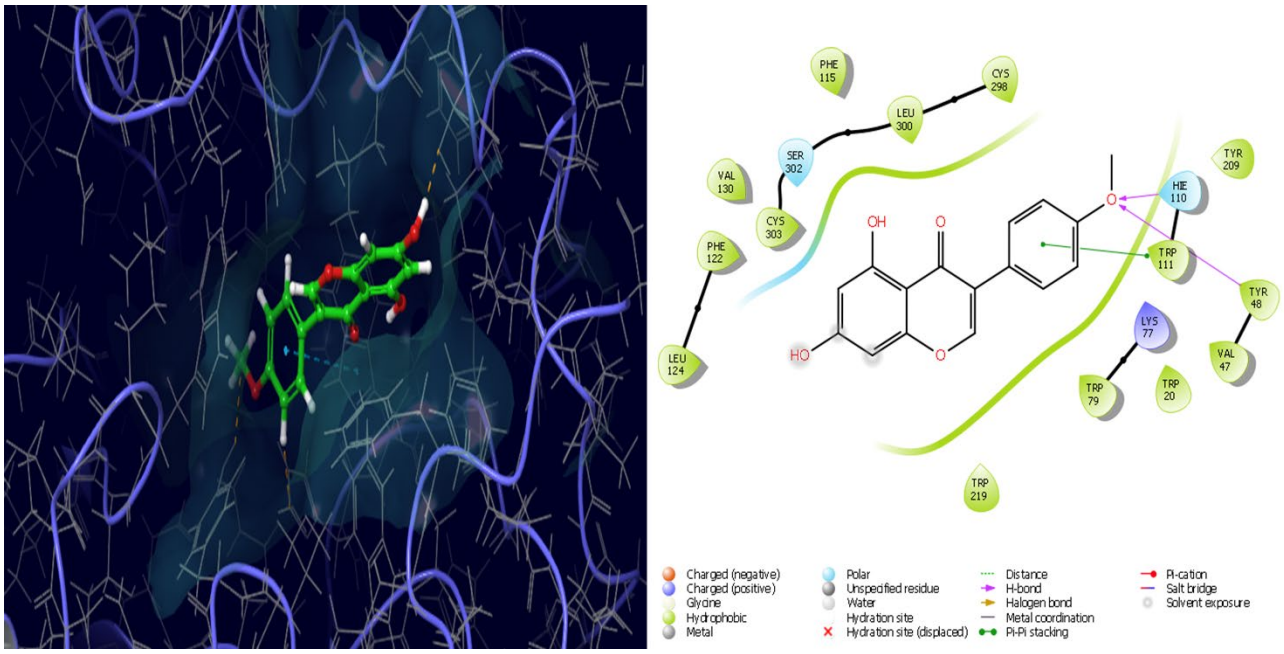

Figure 1. 3D binding mode (left) and 2D ligand interactions (right) of Biochanin A compound with AR enzyme. Ligand binding site represented as a solid surface.

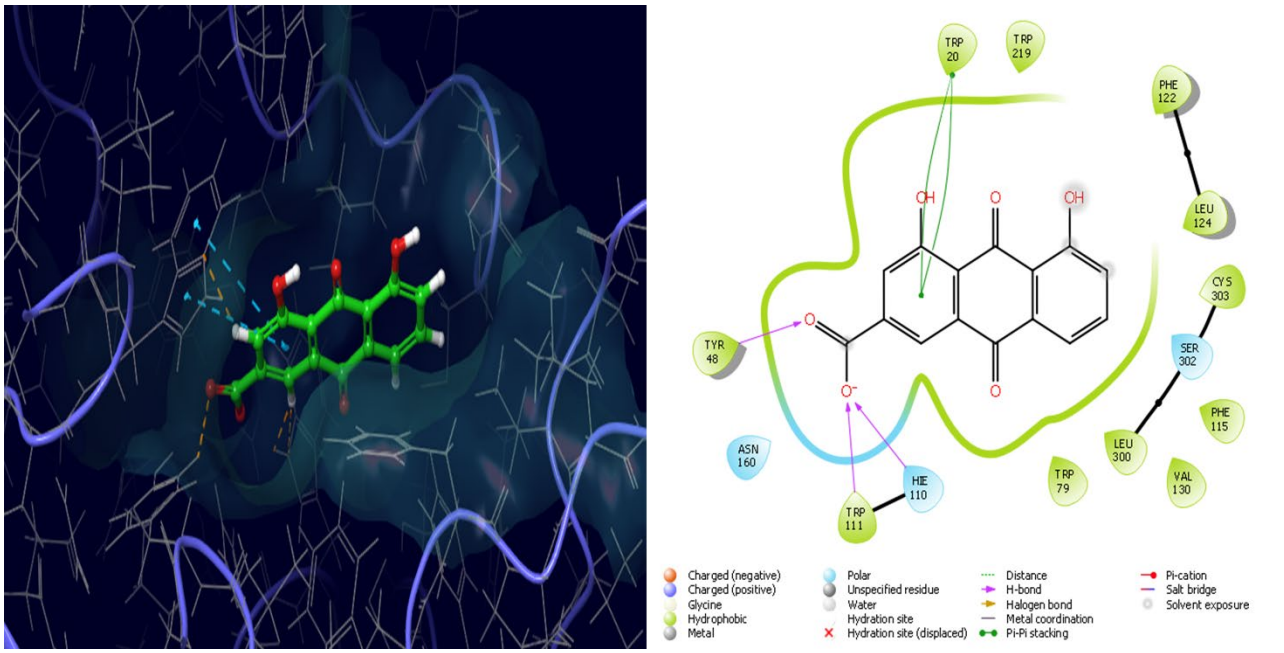

Figure 2. 3D binding mode (left) and 2D ligand interactions (right) of Rhein compound with AR enzyme. Ligand binding site represented as a solid surface.

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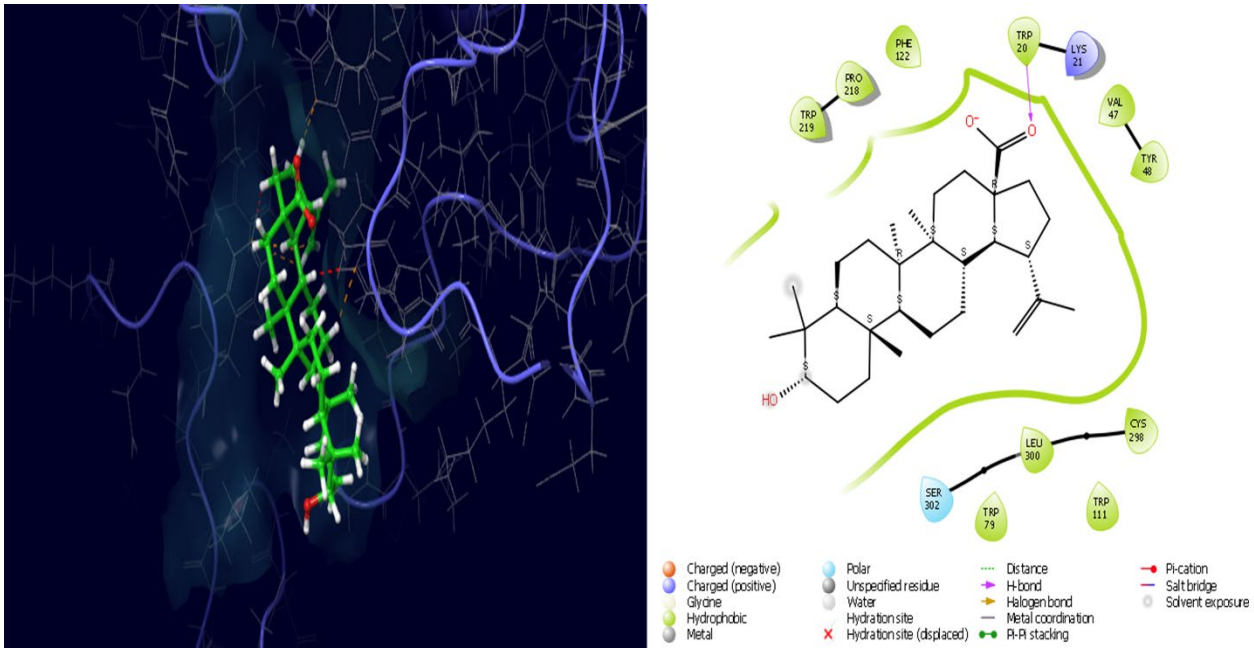

Figure 3. 3D binding mode (left) and 2D ligand interactions (right) of Betulinic acid compound with AR enzyme. Ligand binding site represented as a solid surface.

CONCLUSIONS

Inhibition of the AR enzyme, an enzyme of the polyol pathway that is activated in the case of high blood glucose levels, is vital in terms of preventing and delaying diabetic complications. Thus, the discovery of effective AR enzyme inhibitors is a hope for the treatment of diabetes mellitus-related diseases. Therefore, the *in vitro* inhibition effects of natural antiproliferative agents with known biological activities on the AR enzyme were examined and molecular docking studies were carried out to confirm the experimental inhibition results. Both the obtained experimental data and computer-aided modeling studies showed that all antiproliferative agents whose inhibition effects were examined are good drug candidates for the prevention of diabetic complications.

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Conflict of interests

The author declares that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. El-Kabbani, O.; Ruiz, F.; Darmanin, C.; Chung, R. T. *Cell Mol Life Sci*. **2004**, 61(7), 750-762.

2. Brownlee, M. Nature. **2001**, 414(6865), 813-820.

3. Nishimura, C.; Yamaoka, T.; Mizutani, M.; Yamashita, K.; Akera, T.; Tanimoto, T. *Biochim. Biophys. Acta.* **1991**, 1078(2), 171-178.

4. Hotta, N.; Kawamori, R.; Fukuda, M.; Shigeta, Y. *Diabet. Med*. **2012**, 29(12), 1529-1533.

5. Ramana, K.V.; Srivastava, S.K. *Cytokine*. **2006**, 36, 115-122.

6. Ramasamy, R.; Liu, H.; Oates, P.J.; Schaefer, S. Cardiovasc. Res. **1999**, 42, 130-139.

7. Hwang, Y.C.; Sato, S.; Tsai, J.Y.; Yan, S.; Bakr, S.; Zhang, H.; Ramasamy, R. *FASEB J*. **2002**, 16, 243-245.

8. Berry, G.T. *Eur. J. Pediatr.* **1995**, 154, 53-64.

9. Lee, K.W.; Ko, B.C.; Jiang, Z.; Cao, D.; Chung, S.S. *Anti-cancer drugs*. **2001**, 12, 129-132.

10. Regenold, W.T.; Kling, M.A.; Hauser, P. *Psychoneuroendocrinology*. **2000**, 25, 593-606.

11. Regenold, W.T.; Phatak, P.; Kling, M.A.; Hauser, P. *Mol. Psychiatry.* **2004**, 9, 731.

12. Saraswat, M.; Mrudula, T.; Kumar, P.U.; Suneetha, A.; Rao, T.S.; Srinivasulu, M.; Reddy, G.B. *Med. Sci. Monit.* **2006**, 12, 525-529.

13. Ramana, K.V.; Bhatnagar, A.; Srivastava, S.K. *FASEB J* **2004**, 18, 1209–1218

DOI: http://dx.doi.org/10.32571/ijct.944049 E-ISSN: 2602-277X

14. Chandra, D.; Ramana, K.V.; Friedrich, B.; Srivastava, S.; Bhatnagar, A.; Srivastava, S.K. *Chem. Biol. Interact.* **2003**, 143–144, 605–612

15. Cerelli, K.J.; Curtis, D.L.; Dunn, J.P.; Nelson, P.H.; Peak, T.M.; Waterbury, L.D. *J. Med. Chem.* **1986**, 29, 2347-2351.

16. Schrödinger Release 2020-3: Schrödinger, **2020**, LLC, New York, NY.

17. Sastry, G.M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. *J. Comput. Aided Mol. Des*. **2013**, 27, 221-234.

18. Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Shenkin, P.S. *J. Med. Chem.* **2004**, 47, 1739-1749.

19. Halgren, T.A.; Murphy, R.B.; Friesner, R.A.; Beard, H.S.; Frye, L.L.; Pollard, W.T.; Banks, J.L. *J. Med. Chem.* **2004**, 47, 1750-1759.

20. Genheden, S.; Ryde, U. *Expert. Opin. Drug. Discov.* **2015**, 10(5), 449-461.

21. Prime, Schrödinger, **2020**, LLC, New York, NY.

22. Kato, A.; Yasuko, H.; Goto, H.; Hollinshead, J.; Nash, R. J.; Adachi, I. *Phytomedicine*. **2009,** 16(2-3), 258-261.

23. Ziegler, D. *Nephrol. Dial. Transplant.* **2004**, 19(9), 2170-2175.

24. Sarfraz, A.; Javeed, M.; Shah, M. A.; Hussain, G.; Shafiq, N.; Sarfraz, I.; Riaz, A.; Sadiqa, A.; Zara, R.; Zafar, S.; Kanwal, L.; Sarker, S.D.; Rasul, A. *Sci. Total Environ*. **2020**, 722, 137907.

25. Alakurtti, S.; Mäkelä, T.; Koskimies, S.; Yli-Kauhaluoma, J. *Eur. J. Pharm. Sci*. **2006**, 29(1), 1-13.

26. Zhou, Y. X.; Xia, W.; Yue, W.; Peng, C.; Rahman, K.; Zhang, H. *Evid. Based Complement. Alternat. Med.* **2015**, 2015, 1-10.0