Pharmacokinetic and molecular docking studies of natural plant compounds of *Hibiscus sabdariffa* to design antihypertensive compounds targeting AT2R

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Abstract: AT2R is one of the component of renin-angiotensin system (RAS) plays a role in mediating anti-proliferation, vasodilatation, cellular differentiation, and apoptosis. In the present study natural plant compound, delphinidin 3-O-β-sambubioside delphinidin-3-O-glucoside and cyanidin-3-sambubioside which are anthocyanins, members of the flavonoid group were exploited for controlling the action of AT2R as these are natural colorant found in Hibiscus and exhibit antihypertensive properties. All the selected compounds showed a good binding affinity with AT2R. Delphinidin 3-O-β-sambubioside, delphinidin-3-O-glucoside and cyanidin-3-sambubioside shows the binding affinity of –8.2 kcal/mol, –8.4 kcal/mol and –9.0 kcal/mol respectively with AT2R. Also, the physicochemical properties of these compounds were calculated computationally. The standard drug for AT2R, i.e., compound 21 (C21) shown a binding affinity of –9.1 kcal/mol which is almost similar to that of these compounds. Overall, this study provides a set of lead molecules that can be further explored through in vitro and in vivo experiments for the development of potential drugs against hypertension targeting AT2R.

Keywords: angiotensin type 2 receptor; delphinidin 3-O-β-sambubioside; cyanidin-3-sambubioside; *Hibiscus sabdariffa*; delphinidin-3-O-glucoside; renin-angiotensin system.

Reference to this paper should be made as follows: Sharma, B. (2021) 'Pharmacokinetic and molecular docking studies of natural plant compounds of *Hibiscus sabdariffa* to design antihypertensive compounds targeting AT2R', *Int. J. Computational Biology and Drug Design*, Vol. 14, No. 1, pp.32–42.

Biographical notes: Bhanu Sharma earned his MTech in Biotechnology from the Shoolini University of Biotechnology and Management Sciences, Solan, India. His current research focuses on the use of computational biology tools for finding novel drug targets and designing of new therapeutics.

1 Introduction

Hypertension affects about 1 billion persons worldwide and about one-third of adults alone in the USA suffer from hypertension. Targets that are ideal for control of blood pressure are complex and uncontrolled hypertension increases the threat associated with

diseases of the cardiovascular system (Group et al., 2015). About 6% of death worldwide occurred due to an increase in the pressure of blood in arteries and it is regarded as the most common treatable issue for diseases of the cardiovascular system (Murray and Lopez, 1997; Leeder et al., 2004). In India hypertension is mainly accountable for about 57% of deaths caused by stoke and 24% by coronary heart diseases (Gupta, 2004). The renin-angiotensin system (RAS) plays key functioning of homeostasis maintenance of the body, regulating salt, water, and vascular tone. The main part of the system is Angiotensin II (Ang II) and it exhibits its effects by modulating two major types of receptors i.e., Angiotensin II Type 1 (AT1R) receptor and Type 2 receptor (AT2R) (Clin et al., 1989). The functioning of AT2R was less understood but recent studies shows that AT2R acting a role in cellular differentiation, growth, and opposes AT1R functions (DeGasparo et al., 2000).

In humans, AT1R mediate its biological action when Ang II binds to it and upon activation it perform several functions such as absorption of sodium from renal tubules, vasoconstriction, secretion of aldosterone hormone and the growth of vascular cells (Timmermans et al., 1993). When Ang II interacts with AT2R it counteracts the effects produced by AT1R. AT2R plays two major roles i.e., the regulations of physiological functioning of the kidney, repair and protection of kidney diseases by means of endogenous mechanism (Chow and Allen, 2016). In humans AT2R gene is present on X chromosome at a locus Xq23- 26 and AT2R gene contains three exons, two introns and only exon 3 codes for the AT2R protein (Alfakih et al., 2005; Martin and Elton, 1995). During fetus life, AT2R expressed richly and perform major functions in the morphogenesis of early urinary tract and kidney. AT2R present poorly in tissues of adults except for adrenal medulla, brain, atretic ovary (Niimura et al., 2006). AT2R belongs to the receptor family of G- protein-coupled receptors (Horiuchi et al., 2012). By the stimulation of AT2R, several effects that are produced by AT1R are counteracted by increasing vasodilatation, decreasing the oxidative level of stress and inhibiting the cell proliferation (Jones et al., 2003). Hence the maintenance of balance between the functioning of these receptors (i.e., AT1R and AT2R) is very much useful to maintain proper functioning of the cardiovascular and renal system. The variations in the expression of AT2R and AT1R genes could perform a significant role in the development of diseases related to the cardiovascular system and hypertension (Gribouval et al., 2005). The importance of AT2R in diverse biological functioning will itself made it as a potential drug target and there is need to increase our arsenal of effective drugs and calls for the development of novel therapeutic approaches.

Hibiscus sabdariffa a class of medicinal plant categorised under the family Malvaceae and useful for the medication of hypertension in the indigenous system (Abat et al., 2017). Aqueous extract preparation of this medicinal plant has enormous use in the traditional medicinal system for the medication related to liver diseases, gastrointestinal disorders and hypertension (Monroy-Ortiz et al., 2007). Previously done phytochemical analysis of plant shows the occurrence of organic acids, sterols, phenolics, polysaccharides, terpenoids, and few minerals. Delphinidin-3-O-glucoside, cyanidin-3-O sambubioside, and delphinidin-3-O-sambubioside are mainly contained within the phenolics constituent of the plant (Ali et al., 2005). Therefore, these compounds have a lot of scopes and potential to explore them for possible medication of hypertension. Till date the Compound 21(C21) which is synthesised by modifying nonselective AT1

and AT2 receptor agonist L-162,313 (1) is acting as a highly selective AT2R agonist (Wan et al., 2004).

Docking is very useful and one of the most commonly used methods in structure-based drug design, due to its capability to predict the binding-conformation of small ligand molecules to the appropriate target binding site (Hernández Prada et al., 2008; Chen et al., 2018). In the current study the cyanidin-3-Osambubioside, delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside were screened against AT2R protein. These molecules from docking study were also subjected to protein-ligand interaction studies and prediction of pharmacokinetic properties. These molecules can be strongly suggested for further wet lab (*in vitro and in vivo*) experiments for drug design and discovery.

2 Materials and methods

2.1 Targeted angiotensin II type 2 receptor

The structure of human AT2R was retrieved with PDB ID 5UNF from the RCSB protein data bank. The resolution of the 3D structure of the AT2R was 2.8 Å (Zhang et al., 2017). The already bound ligand molecule was removed from active site before docking. Also for docking studies the protein AT2R was assigned with polaraties, calculating Gasteiger charges and hydrogen. In the end final protein is ready for docking by converting protein structures from the pdb format to pdbqt format using Auto-Dock tool1.5.4.

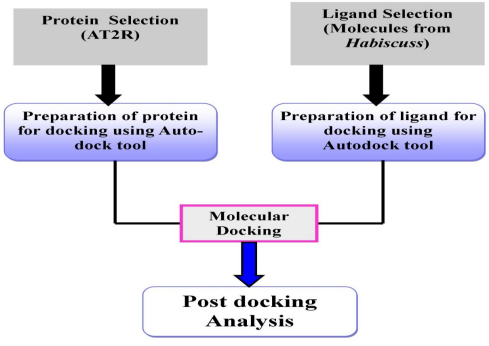
2.2 Ligand preparation of cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside

The chemical structures for the phytoconstituents cyanidin-3-Osambubioside, delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside which are found in the extract of *Hibiscus sabdariffa* were retrieved from pubchem database (Ojeda et al., 2010). These downloaded files were optimised for the purpose of molecular docking and subsequently converted to pdbqt format using Autodock tools 1.5.6.

2.3 Molecular docking analysis of angiotensin II type 2 Receptor

By performing the molecular docking with the help of autodock vina the potential application of the compounds cyanidin-3-Osambubioside, delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside was evaluated against AT2R which is a part of the RAS system (Figure 1). The grid was set manually examining the protein structures so that it can surround the region of interest (active site) (Seeliger et al., 2010). The criteria which were used for elucidating the inhibitory potential of ligands were the binding energies of ligands, binding pose, hydrogen bonding and hydrophobic interactions with the receptor. Lesser the binding energy of ligand with the receptor more affinity is shown by the ligand for that particular receptor. After the molecular study has been done, UCSF chimera 1.9 software was used to study and visualise the resultant receptor-ligand complexes (Pettersen et al., 2004).

Figure 1 Protocol followed for docking (see online version for colours)



2.4 Validation of computational molecular docking

For the accuracy of molecular docking, redocking of cocrystallised ligand onto the active site of AT2R was done. Root-mean-square deviation (RMSD) between the original conformation and docked structure of the inhibitor in each complex was calculated (Dhananjayan, 2015).

2.5 ADME-Toxicity prediction

The pharmacokinetic properties of these organic compounds were done by using ADMETSAR server (http://lmmd.ecust.edu.cn:8000/predict/), with incorporated prediction of toxicity, metabolism, distribution, absorption and excretion (Cheng et al., 2012).

3 Results

In the present study, considering the importance of AT2R protein as a drug target for hypertension molecular docking was carried out. The organic molecules from *Hibiscus sabdariffa* were docked into the active site of AT2R to exploit the potential of these molecules which can be further in near future utilised for drug design experiments. Pharmacokinetics was also calculated computationally to check the drug like properties of these molecules.

3.1 Validation of molecular docking

The methodology used for docking was validated by using validation approach in order to determine the reliability of molecular docking. RMSD between the original conformation and docked structure of the inhibitor was calculated. If the RMSD of the docked pose is found to be equal or less than 1.0 Å from the experimentally observed conformation then the prediction is considered as successful (Table 1).

Table 1 Validation set showing the docking score of known inhibitors onto the crystal structure of AT2R

Known inhibitor	*PDB*	*Resolution of AT2R Å*	*RMSD Å*	*Docking score kcal/mol*
8ES	5UNF	2.8	0.94	–11.0

Table 2 Binding affinities (kcal/mol) of cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside with AT2R

S. No.	*Plant*	*Ligand*	*Binding affinity* (kcal/mol)	*Amino Acid*	*Bond Length (Å)*	*Bond Formed*
1	*Habiscus sabdariffa*	Cyanidin-3-O-sambubioside	–9.0	LYS215	3.07	H-bonding
				TYR204	3.14	
				ARG182	2.95	
				MET197	2.91	
				TRP100	3.05	
2	*Habiscus sabdariffa*	Delphinidin-3-O-sambubioside	–8.2	ARG182	3.26,3.17	H-bonding
3	*Habiscus sabdariffa*	Delphinidin-3-O-glucoside	–8.4	ARG 182	3.48	H-Bonding
4	POSITIVE CONTROL	C21	–9.1	THR125	3.01	H-Bonding
				THR178	3.06	
				ARG182	3.23,2.93	

3.2 Molecular docking analysis

The AT2R was successfully docked with the organic compounds cyanidin-3-O-sambubioside, delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside. The binding affinities of the ligands with AT2R were analysed; cyanidin-3-O-sambubioside shows the minimum energy value of –9.0 kcal/mol, delphinidin-3-O-sambubioside shows the minimum energy value of –8.2 kcal/mol and delphinidin-3-O-glucoside shows the binding affinity of –8.4 kcal/mol (Table 2). Out of the three compounds cyanidin-3-O-sambubioside shows the best binding affinity with the AT2R. The 2D interaction pattern of cyanidin-3-O-sambubioside generated by ligplot has been shown in Figure 2 and structure of 3D interactions has been shown in Figure 3. Angiotensin II type 2 receptor residues ARG182, MET197, TYR204, LYS215 and TRP100 were form hydrogen bonds with the cyanidin-3-O-sambubioside and the affinity value of compound was –9.0 kcal/mol. The ligplot generated binding for the delphinidin-3-O-sambubioside has been shown in Figure 4 and 3D structure of interactions has been shown in Figure 5. AT2R residue ARG182 was form hydrogen bond with the delphinidin-3-O-sambubioside and affinity value obtained was –8.2 kcal/mol. The other compound delphinidin-3-O-glucoside shows the binding affinity of –8.4 kcal/mol and strongly interacts with ARG 182. The positive control used is C21 is a synthetic compound as a standard shows the affinity value of –9.1 with AT2R and residues which participate in hydrogen bonding were THR125, THR172 and ARG182 (Table 2). The ligplot generated for the binding of

AT2R with C21 has been shown in Figure 6 and 3D structure of interactions is shown in Figure 7.

3.3 ADME-Toxicity prediction

ADMET of compounds is important to identify their drug likeliness properties and it is obligatory to check whether the compounds were toxic or. Important parameters of ADMET for these compounds were presented in the Table 3.

Figure 2 Interaction of cyanidin-3-O-sambubioside with AT2R generated by Ligplot (see online version for colours)

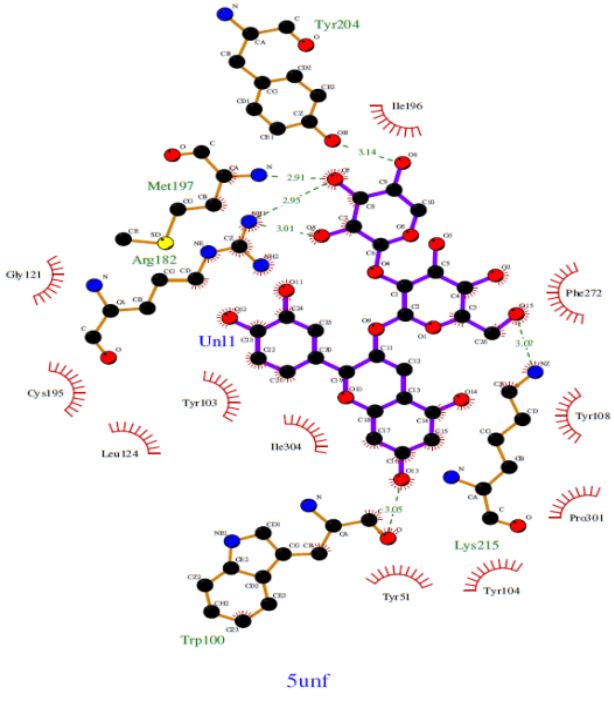


Figure 3 Cyanidin-3-O-sambubioside with AT2R with grid box centre values $X = 77.18$, $Y = 3.76$, $Z = 36.162$ with dimension 40 (see online version for colours)

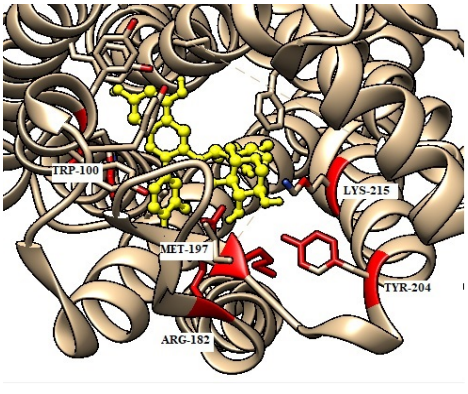


Figure 4 Interaction of delphinidin-3-O-sambubioside with AT2R generated by Ligplot (see online version for colours)

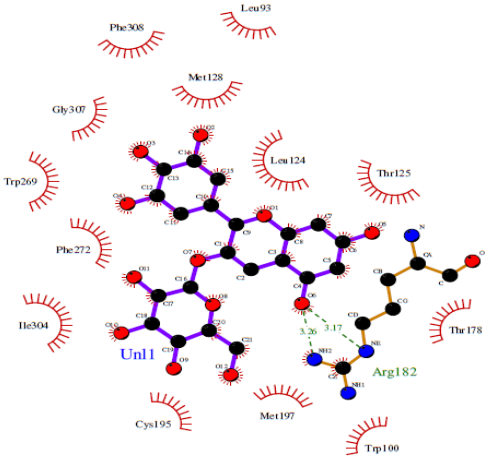


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Figure 5 Delphinidin-3-O-sambubioside with AT2R with grid box centre values $X = 77.969$, $Y = -0.016$, $Z = 34.654$ with dimension 40 (see online version for colours)

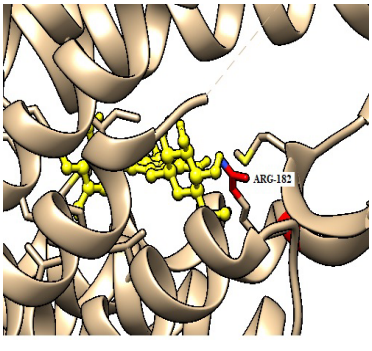

Figure 6 Interaction of C21 with AT2R generated by Ligplot (see online version for colours)

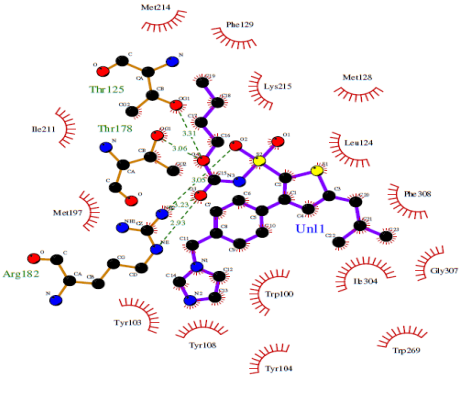

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Figure 7 C21 with AT2R with grid box centre $X = 77.969$, $Y = –0.016$, $Z = 34.654$ with dimension 40 (see online version for colours)

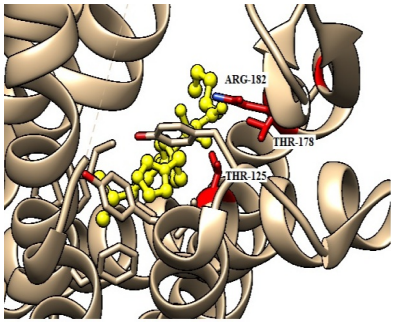

Table 3 ADMET parameters of compounds of *Hibiscus sabdariffa*

Parameters	*Cyanidin 3-sambubioside*	*Delphinidin 3-sambubioside*	*Delphinidin–3-O-glucoside*
Human Ether-a-gogo-Related Gene Inhibition	WI	WI	WI
AMES Toxicity	NAT	NAT	NAT
Carcinogens	NC	NC	NC
Honey Bee Toxicity	HHBT	HHBT	HHBT
Biodegradation	NRD	NRD	NRD
CYP450 2C9 Substrate	NI	NI	NI
CYP450 2CD6 Substrate	NI	NI	NI
Human Intestinal Absorption	HIA(0.765)	HIA(0.8643)	HIA(0.832)
Molecular weight	581	597	465.4
Log P	–1.1547	–1.44	0.0876

NC = NON TOXIC, NI = Non Inhibitor, NRD = Not Readily Degraded, HHBT = High Honey bee toxicity, NAT = Non Ames Toxic, WI = weak inhibitor.

4 Discussion

Computational techniques are widely exploited in recent drug designing purposes for better visualisation and understanding of the drug-receptor interaction. It has been suggested in many studies that the molecular docking can play important role for design of more potential, novel inhibitors by enlightening the mechanism of drug-receptor interaction (Kellici et al., 2015; Paliwal et al., 2012).

Over hundreds of years, the RAS was considered to play a major role in maintaining the arterial pressure, sodium and fluid homeostasis (Siragy, 2004). The AT2R these days with MAS receptor regarded as main receptors which act as a protective arm of the renin-angiotensin system (Chow and Allen, 2016). The major role which is performed by the AT2R when it got activated by the Ang II is maintaining the blood pressure in heart, kidney and blood vessels (Oboh et al., 2014).

AT2R non-peptide drug that is C21 is used as an agonist which enhance AT2R activity shown in many clinical trials and *in vivo* studies. In recent studies, Hallberg et al. demonstrated many protective effects of AT2R like stimulation of AT2R can lower blood pressure in Ang II-induced hypertension (Hallberg et al., 2018). These findings show that it is a potent target for the medication of hypertension but C21 is a synthetic compound which can be replaced by natural medicines. In recent decade many of the studies and researchers focus on the use of natural bioactive compounds to find suitable medications for many diseases. Ojeda et al. in their findings elucidate the *Hibiscus sabdariffa* isolated compounds cyanidin-3-O-sambubiosides and delphinidin-3-O-sambubioside has antihypertensive properties but exact mechanism was not known. These two molecules show good competitive inhibition property against ACE (Ojeda et al., 2010).

Hence in present investigation molecular docking of AT2R with cyanidin-3-O-sambubioside, delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside was utilised to explore the potential of these organic molecules against another component of RAS i.e., AT2R. The molecular docking of AT2R with these organic compounds shows good intermolecular hydrogen bonding, stacking interactions and having computationally predicted low energy complexes. Cyanidin-3-O-sambubioside shows lower energy complex as compared to the delphinidin-3-O-glucoside and delphinidin-3-O-sambubioside. Hence, these molecules can be used as a potent natural compound for targeting AT2R and in near future can be utilised to design suitable therapeutics (agonist or antagonist molecules) for hypertension. The natural bioactive compound cyanidin-3-O-sambubioside shows almost similar interactions with AT2R when compared with standard i.e., C21 (Table 2) and hence can be used as lead molecule for drug design. Determining toxicity of compounds is one of the major aspects of drug design. The ADMET values also predicts that the organic molecule cyanidin-3-O-sambubioside have good drug likeliness properties which also support to use cyanidin-3-O-sambubioside for further drug designing.

5 Conclusion

The natural compound obtained from *Hibiscus sabdariffa* i.e., Cyanidin-3-O-sambubioside had shown the binding affinity of –9.0 kcal/mol with AT2R while, standard drug C21 had –9.1 kcal/mol which almost similar and it indicates that this compound can be used for the treatment of hypertension instead of synthetic compound C21 by stimulating AT2R. Docking score, interaction and ADMET properties of top Cyanidin-3-O-sambubioside was found to be favourable to pursue further drug development for curing hypertension by targeting AT2R. Overall the current findings provide a suitable starting point for further *in vitro* and *in vivo* analyses to exploit AT2R as a potential drug target for hypertension.

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