Asian Journal of Chemistry; Vol. 30, No. 12 (2018), 2693-2698

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21550

in silico Studies on Components of *Murraya koenigii* as Activators of Insulin Receptor

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Received: 28 June 2018; *Accepted*: 31 August 2018; *Published online*: 31 October 2018; AJC-19133

In this study, components of *Murraya koenigii* are subjected to molecular docking studies targeting insulin receptor in order to develop a new lead for diabetes. Initially among all the ligands best one is identified by docking analysis using CCDC GOLD software and further analogs are prepared for it. In the present study we have used commercial computational tools like Accelrys Discovery Studio 2.5 to identify the novel analogs from the docking studies and their ADME (absorption, distribution, metabolism and excretion) and toxicity profiles were studied using *in silico* tools. We found that among all the ligands girnimbine is having a high dock score and higher interaction energy, so analogs were prepared for it. Computational analysis of the results showed that the substitution of carboxy ethyl and hydroxyl groups at R_1 and R_2 positions on the side chains of girnimbine showed higher dock score and interaction energy than girnimbine and their corresponding molecular properties, ADME and toxicity profiles were also studied using *in silico* tools.

Keywords: Molecular docking, Diabetes, *Murraya koenigii,* Girnimbine, Insulin receptor.

INTRODUCTION

Murraya koenigii is an aromatic more or less deciduous shrub belonging to the class eudicots and family Rutaceae which is native to India and Srilanka [1]. It originated in the Tarai region of Uttar Pradesh, India and at present it is cultivated in Burma, Ceylon, China, Australia and Pacific Islands [2]. *Murraya koenigii* is used as folk medicine for the treatment of various metabolic and infectious diseases. In the present study, *Murraya koenigii* was chosen since it is one of the most widely acclaimed remedies for the treatment of diabetes [3]. Diabetes mellitus is a debilitating and often life threatening disease with increasing incidence throughout the world. The prevalence of diabetes was also found to be increasing rapidly in rural areas, as a result of the recent socio-economic transitions [4]. A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and therapeutic strategies. Alternatives are clearly needed because of the high cost and poor availability of current therapies for many rural populations particularly in developing countries [5]. The essential oils from the leaves of *M. koenigii* analyzed by gas chromatography and mass spectroscopy presented that it contained 39 compounds of which the major is 3-carene (54.22 %) followed by caryophyllene (9.49 %) [6-8]. The stem bark and roots of *M. koenigii* were extracted with hexane, chloroform and methanol. The fractionation of hexane and chloroform extracts followed by column chromatography and TLC yield carbazole alkaloids which were identified as girnimbine, murrayafoline-A and murrayanine by spectroscopic methods [9]. Carbazole alkaloids present in *M. koenigii* leaves were reported to have antioxidant and antidiabetic activities. Biological activities of *M. koenigii* leaves were also reported for anti-hypercholesterolemic as well as for their efficacy against colon carcinogensis [10-13].

The present study aimed to design and identify the potent activator for the insulin receptor (protein) from the above mentioned compounds which we call as ligands in docking. Structure-based drug design relies on knowledge of the three dimensional structure of the biological target obtained through methods such as X-ray crystallography or NMR spectroscopy [14]. These approaches can be used for generating diverse molecular structures [15,16]. Using the structure of the biological target, candidate drugs that are predicted to bind with the target may be designed [17]. This drug design frequently but not necessarily relies on computer modeling techniques [18]. The interactions of these ligands with the protein by using

GOLD (generic optimization for ligand docking) docking protocol were studied and analogs were designed for the best fitted ligand. The designed analogs were subjected for docking with the protein and results of analogs are compared to best fitted ligand to find out the best analog which acts as potent activator for insulin receptor. Simultaneously the molecular properties, ADME and toxicity profile for both ligands and analogs were also studied. These QSAR relationships in turn may be used to predict the activity of new analogs [19]. The key advantage of such a method is that novel structures, not contained in any database, can be suggested [20].

EXPERIMENTAL

The experiment employed to figure out the potential ligands is molecular docking using various computational softwares like Hyperchem, Drug discovery studio, SPDBV, GOLD. The target protein and the ligands selected are insulin receptor and components of *Murraya koenigii* (carene, caryophyllene, girnimbine, murrayafoline-A and murrayanine respectively). The ligands are made to interact with the target protein using software tools and various parameters determining the strength of interactions are calculated. Ligands are ranked based on the degree of fitness and binding energy. The following are the softwares employed to perform our study.

HyperChem professional 8.0 is a versatile molecular modeller and editor and a powerful computational package. It offers many types of molecular and quantum mechanics calculations. For optimization of small molecules in solution and protein complex the intra molecular energies of ligand-solvent and ligand protein will be calculated using molecular mechanics calculations of Hyperchem software.

Accelrys has released Discovery Studio 2.5, an advanced computational chemistry and biology software environment for drug discovery. It can be used for many processes such as ligand optimization, protein optimization, calculation of molecular properties, prediction of ADME and toxicological parameters. The accuracy of the software is very best when compared with various other software such as Auto Dock, HEX and ARGUS *etc*.

GOLD (genetic optimization for ligand docking) uses genetic algorithm to provide docking of flexible ligand and a protein with flexible hydroxyl groups. This makes it a good choice when the binding pocket contains amino acids that form hydrogen bonds with the ligand. GOLD offers a choice of scoring functions: GoldScore, ChemScore and User Defined Score. The solutions are known to have 70-80 % accuracy when tested on complexes extracted from Potein databank (PDB). The version used is CCDC Gold Suite 4.12.

Schrödinger software suite is drug design software using both ligand and structure-based methods. Schrödinger provides accurate, reliable and high performance computational technology to solve real-world problems in life science research. The predictive power of Schrödinger's software allows scientists to accelerate their research and development activities, reduce research costs and make novel discoveries that might not be possible with other computational or experimental approaches. QikProp tool in schrodinger is a quick, accurate, easy-to-use absorption, distribution, metabolism and excretion (ADME) prediction program. It predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches.

Step-1: Among the various components present in *M. koenigii* the ligands were selected based on Lipinski's rule of five which is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. Lipinski's Rule of Five was then applied to select probable ligands. Those compounds that had more than one violation were eliminated.

Step-2: The selected lead compounds were optimized in a step wise process using computational methods in combination with SAR information to determine areas on the molecule to expand, contract, or modify. Models of the ligands were built using HyperChem 8.0. The force field set was Amber (parameter A2). According to the IUPAC nomenclature the back bone of the structure was drawn. Using the "add H and model build" control, a complete model (unoptimized) was built. Single point energy and gradient was calculated using the control, "single point" under compute. The molecule so formed is saved in ".mol" format.

Step-3: Molecular dynamics is carried out to anneal the system to obtain a lower energy minimum. They calculate the future positions and velocities of atoms based upon their current values. A dynamics run has three optional phases: heat, run and cool. The first phase occurs over a simulation period of heat time, using the starting temperature to set initial velocities with rescaling of velocities at temperature increments to reach the simulation temperature. In the middle phase, velocities are rescaled only if constant temperature is selected. The final phase occurs over a simulation period of cool time, with rescaling of velocities at temperature increments to reach the final temperature. The simulations (molecular dynamics, langevin dynamics, MonteCarlo simulations) are carried out cautiously and the values obtained are carefully documented along with the snapshots. This step is performed using the software HyperChem 8.0. Each simulation is performed starting with the geometrically optimized molecule obtained in step-1 and independent of the other, with the dynamics run in no particular order.

Step-4: The drug and its analogs stored in .mol format were handled using Discovery Studio software. A force was set up for the molecule (charm27) and checked whether all the molecules are typed or not. Energy and geometry optimization for each and every molecule was performed using the control, "minimization", under the options Protocols and Simulation. The reports were saved and the resultant optimized/minimized molecules were saved in .mol2 format which is accessible for many softwares such as GOLD DOCK, ARGUS, SCHRODINGER *etc*. Various properties of a drug such as molecular, ADME and toxicological properties were accurately predicted and results were saved in respective formats like. csv,. mol,. sd and. pdf.

Step-5: The RSCB Protein Data Bank was searched for the insulin receptor (1IR3) and downloaded in .pdb format and was loaded in Discovery Studio. Protein report was checked. Solvent molecules were removed and hydrogen's were added.

Hetero atoms were also removed. Missing/incorrectly specified residues were corrected. A force field (charm 27) was applied and the molecules which were not typed are manually selected and assigned a force field. Energy and geometry optimization for each and every molecule was performed using the control, "minimization", under the options Protocols and Simulation. Active site analysis of the Insulin Receptor was carried out using Swiss PDB Viewer (SPDBV) V.4.02 and from the PDB ligand Explorer. In order to pin point the location of the active site in the protein we have used the assistance of SPDBV software. The active site consists of five residues and can be seen in Fig. 1, amino acid residue number and the active site number are mentioned in Table-1.

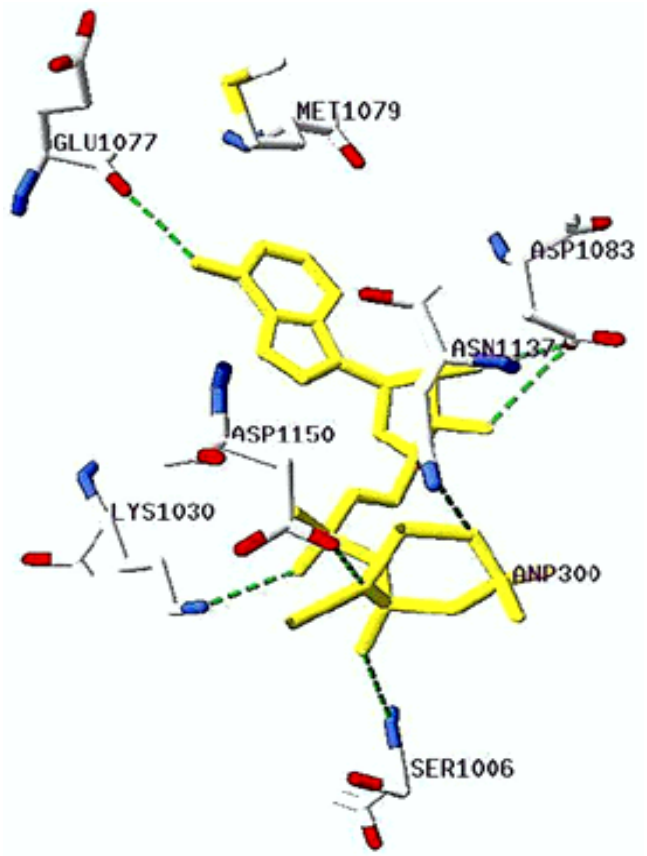


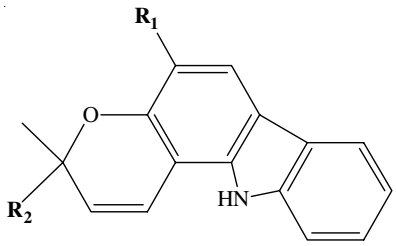
Fig. 1. Active site analysis-the yellow stick model represents the active site of insulin receptor

TABLE-1

Amino acid	Residue number	Active site number
Glu	1077	1459
Met	1079	1493
Asp	1083	1544
Lys	1030	738
Ser	1006	400

Step-6: Docking procedure is performed using CCDC GOLD suite software. GOLD uses a generic algorithm to calculate the binding energies which determine the activity of the molecules when bound to the protein molecule. The minimized protein previously saved in mol2 or pdb format (step-6) is loaded into the software. After loading the minimized protein, the binding site is defined by giving the residue number in the binding site field. The rest of the fields are left alone as they were taken care off in step-5. Later, ligands are browsed and loaded in the software. In the fields, ligand flexibility, fitness and search option and generic algorithm settings, the values are set to default. In atom typing field both protein and ligand options were selected in the output options field the path of the results were specified. The rest of the fields are left to default values. Finally, the option RUN GOLD has been selected to perform docking.

Step-7: Based on docking fitness results among all the 10 ligands girnimbine was found to have the best fitness for the insulin receptor. The analogs of girnimbine were generated without disturbing the pharmacophore of the molecule, *i.e.*, without removing the essence of girnimbine. The structure activity relationship of girnimbine was observed and modifications are only done at R_1 and R_2 positions which are given in Table-2.



Structure of girnimbine

TABLE-2
MODIFICATIONS OF GIRNIMBINE
FOR PREPARATION OF ANALOGS

Analog number	R_1	R_2
Analog I	CH_3	C_2H_5
Analog II	CH_3	$COOC_2H_5$
Analog III	OCH_3	C_2H_5
Analog IV	OCH_3	$COOC_2H_5$
Analog V	OH	$COOC_2H_5$
Analog VI	NH_2	$COOC_2H_5$
Analog VII	H	$COOC_2H_5$
Analog VIII	$COOC_2H_5$	$COOC_2H_5$
Analog IX	$COOC_2H_5$	OH
Analog X	$COOC_2H_5$	H
Analog XI	NH_2	OH
Analog XII	OH	OCH_3
Analog XIII	OCH_3	NH_2
Analog XIV	OCH_3	$COOCH_3$
Analog XV	OCH_3	H

Step-8: These analogs were initially designed using OSIRIS property explorer which is an integral part of Actelion's in house substance registration system and lets us draw chemical structures and calculates various drug-relevant properties whenever a structure is valid. Prediction results are valued and colour coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red whereas a green colour indicates drug-conform behaviour.

Step-9: The analogs were also subjected for docking process similar like ligands. The drug and its analogs which were stored in .mol format were opened using QIKPROP tool

TABLE-3
DOCKING RESULTS OF LIGANDS

S. No.	Compound name	Fitness	H-bond		van der Waal forces	
			Internal	External	Internal	External
1	3-Carene	30.97	0	0	24.67	-2.95
2	Caryophyllene	32.61	0	0	25.63	-2.63
3	Girnimbine	46.45	0	1.17	33.46	-0.73
4	Murrayafolin	41.16	0	1.82	30.49	-2.58
5	Murrayanine	40.52	0	0.53	30.75	-2.29

of SCHODINGER and various properties of a drug such as molecular, ADME and toxicological properties were accurately predicted. The results were saved in respective formats like .csv, .mol, .sd and .pdf.

RESULTS AND DISCUSSION

Protein and ligand interactions are studied by using GOLD docking software. Results are in format of Gold fitness which comprises of terms:

$$\text{GOLD Fitness} = \text{Shb\_ext} + 1.3750 \times \text{Svdw\_ext} + \text{Shb\_int} + \text{Svdw\_int}$$

where Shb_ext is the protein-ligand hydrogen bond score and Svdw_ext is the protein-ligand van der Waals score. Shb_int is the contribution to the fitness due to intramolecular hydrogen bonds in the ligand and Svdw_int is the contribution due to intramolecular strain in the ligand. Hydrogen bond and van der Waals forces determine the fitness of docked ligand. The ligand which is having the highest fitness score is having the highest binding affinity

Docking results of ligands were given in the Table-3 and it is observed that of all the ligands girnimbine is found to have a good fitness score and shown in Fig. 2. Next to girnimbine the other compounds having better fitness are murrayafolin and murrayanine. For each ligand their respective hydrogen bond external and internal values and van der Waal forces external and internal values were given. The interactions of ligands with targetad protein are analyzed after the docking studies. The results are tabulated in the Table-4.

TABLE-4
HYDROGEN BOND ANALYSIS FOR LIGANDS

Compound name	No. of H-bonds	Amino acid name	H-bond distance
Girnimbine	1	ASP 1150	2.836
Murrayafolin-A	1	ASP 1150	2.741
Murrayanine	1	ASP 1150	2.506
Caryophyllene	1	ASP 1150	2.517
3-Carene	1	ASP 1083	2.410

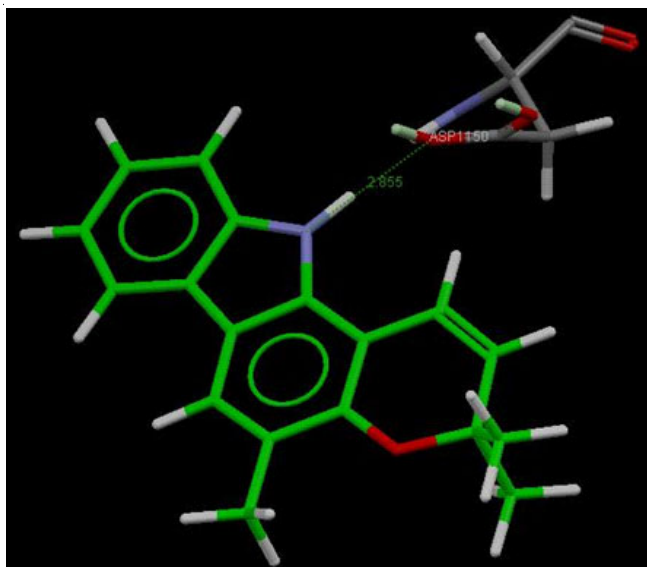


Fig. 2. Binding interaction of girnimbine with protein

The ligand having high fitness score girnimbine is found to have one hydrogen bond with amino acid ASP 1150 where as other ligands are having one hydrogen bond with ASP 1150 and caffeic acid with ASP 1083. Their respective hydrogen bond distances were also reported.

ADME profile, also called as pharmacokinetic profile of the ligands are tabulated in the Table-5. All the ligands have good absorption level. All the ligands have good aqueous solubility and drug like properties except caryophyllene and murrayafolin which are having low. All the ligands does not show BBB penetration except 3-carene and caryophyllene which has low levels of BBB penetration. 3-Carene, caryophyllene and girnimbine do not show hepatotoxicity and they are unlikely to cause dose-dependent liver injuries. All ligands are unlikely to inhibit CYP2D6 enzyme. Except 3-carene all other ligands are having greater than 90 % of plasma protein binding.

Toxicity profiles of ligands are studied by using an Accelery Discovery Studio. TOPKAT models have been re-trained using updated training sets from the legacy TOPKAT (Toxicity Pre-

TABLE-5
ADME PROPERTIES OF THE LIGANDS

Compound name	BBB level	Absorption level	Solubility level	Hepatotoxicity level	CYP2D6 level	PPB level
3-Carene	3	0	-3.67	0	0	0
Caryophyllene	3	1	-5.79	0	0	1
Girnimbine	4	0	-3.79	0	0	2
Murrayafolin-A	4	0	-5.36	1	0	2
Murrayanine	4	0	-4.78	1	0	2

TABLE-6
TOXICITY PROPERTIES OF LIGANDS

Compound name	Aerobic Biodegradability	Mutagenecity	Developmental Toxicity potential	Occular irritancy	Skin irritancy	Carcinogenecity				
						Rodent	Female mouse	Male mouse	Female rat	Male rat
3-Carene	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES
Caryophyllene	YES	NO	YES	YES	NO	YES	YES	YES	YES	YES
Girnimbine	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Murrayafolin-A	NO	YES	NO	YES	YES	YES	NO	YES	NO	YES
Murrayanine	NO	YES	YES	NO	YES	YES	NO	YES	NO	YES

TABLE-7
DOCKING STUDY RESULTS OF ANALOGS

S. No.	Compound name	Fitness	H-bond		van der Waal forces	
			Internal	External	Internal	External
1	Girnimbine	46.45	0.00	1.17	-0.73	33.46
2	Analog (I)	48.66	0.00	0.00	-1.27	38.59
3	Analog (II)	50.65	0.00	0.00	-0.99	36.11
4	Analog (III)	49.31	0.00	0.00	-0.25	37.02
5	Analog (IV)	51.79	0.00	0.00	-1.74	37.13
6	Analog (V)	52.42	0.00	7.66	-1.13	33.38
7	Analog (VI)	50.46	0.00	1.31	-0.13	35.84
8	Analog (VII)	49.97	0.00	0.00	0.22	36.18
9	Analog (VIII)	52.26	0.00	1.49	-3.53	39.50
10	Analog (IX)	49.56	0.00	0.99	-2.87	37.41
11	Analog (X)	48.03	0.00	0.58	-2.33	36.20
12	Analog (XI)	45.65	0.00	1.37	-1.25	33.11
13	Analog (XII)	48.94	0.00	6.93	-2.56	32.41
14	Analog (XIII)	31.11	0.00	1.87	-14.7	31.95
15	Analog (XIV)	49.48	0.00	0.00	-2.50	37.80
16	Analog (XV)	45.78	0.00	0.00	-1.73	34.56

diction by Computer Assisted Technology). The classification models are built with Bayesian models and the regression models are built with the partial least squares (PLS) technique. From the *in silico* toxicity studies, listed in Table-6 we found that except 3-carene and caryophyllene all other ligands are non-biodegradable in aerobic conditions. Mutagenecity was exhibited by murrayafolin-A and murrayanine. Girnimbine and murrayafolin-A have no dose dependent toxicity potential. Except girnimbine and murrayanine all the other ligands show ocular irritancy. 3-Careen, murrayanine and murrayafolin-A exhibited skin irritancy. Except girnimbine we found carcinogenicity for other ligands.

All 16 analogs and girnimbine were docked with protein and docking results are tabulated in the Table-7. Among all the ligands, we observed that analogs (V), (VIII), (IV), (II), (VI), (VII), (IX), (XIV), (III) and (XII) are having the best fitness score and it is more than girnimbine so hydrogen bond analysis was performed for these analogs and it is given in Table-8. For each analog their respective hydrogen bond external and internal values and van der Waal forces external and internal values were given. Of all the analogs analog V is having the highest fitness score and it is given in Fig. 3.

Analog (V) having best fitness score is found to have a hydrogen bond with ASP 1150 and we observe that analog (IV) and (III) are having two hydrogen bonds with ASP 1150 and analog (IV) is having three hydrogen bonds two with ASP 1150 and one with ASP 1083 and analog (VIII) and (XII) are having one hydrogen bond with ASP 1150 and their respective bond distances were given in the Table-8.

TABLE-8
HYDROGEN BOND ANALYSIS FOR ANALOGS

Compound name	No. of H-bonds	Amino acid name	H-bond distance
Analog (V)	1	ASP 1150	2.735
Analog (VIII)	1	ASP 1150	2.986
Analog (IV)	2	ASP 1150	2.953
		ASP 1150	2.690
Analog (XIV)	3	ASP 1150	2.799
		ASP 1150	2.807
		ASP 1083	2.577
Analog (III)	2	ASP 1150	2.725
		ASP 1150	2.698
Analog (XII)	1	ASP 1150	2.883

All the ADME properties were calculated for the analogs and given in Table-9. All the top 10 analogs are found to be CNS inactive. The Caco-2 cell permeability of analogs was found to be great. The QPPlogBB values are negative indicating they are too polar to cross the blood-brain barrier. MDCK (Madin-Darby Canin Kidney) cell permeability was found to be great. All the analogs have less predicted skin permeability. Predicted human oral absorption of top ten analogs was found to be high. Hence by these results we can infer that all the top ten analogs having good fitness score than girnimbine are having good ADME profile.

TABLE-9
ADME PROPERTIES OF ANALOGS

Analog ranking	Analog No.	CNS activity	QPP Caco2	QPP log BB	QPP MDCK	QP log Kp	Human oral absorption (%)
1	Analog (V)	0	1177.49	-0.514	590.37	-2.052	100
2	Analog (VIII)	0	1689.28	-0.456	871.9	-1.698	100
3	Analog (IV)	0	3110.44	-0.102	1686.78	-1.256	100
4	Analog (II)	0	3067.51	-0.065	1661.53	-1.35	100
5	Analog (VI)	-1	1077.42	-0.553	536.23	-2.114	100
6	Analog (VII)	0	3395.7	-0.009	1854.58	-1.123	100
7	Analog (IX)	0	1206.11	-0.53	605.89	-2.015	100
8	Analog (XIV)	0	2620.87	-0.105	1401.58	-1.49	100
9	Analog (III)	1	6392.97	0.265	3674.61	-0.74	100
10	Analog (XII)	0	2166.92	-0.184	1141.29	-1.561	100

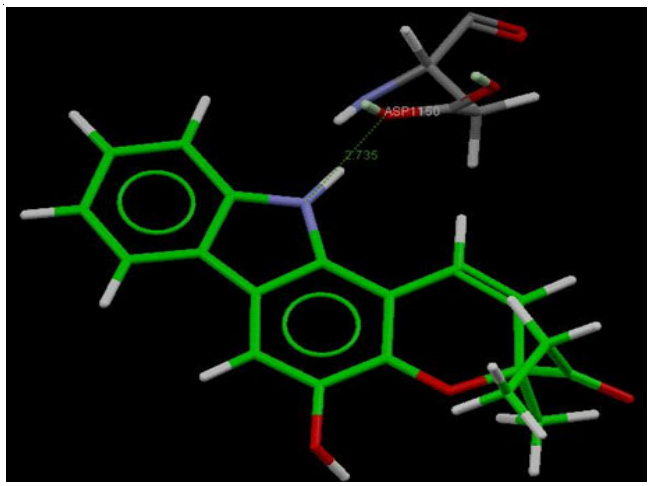


Fig 3. Binding interaction of analog V with protein

Conclusion

In the present study components of *Murraya koenigii* are subjected to molecular docking studies targeting insulin receptor and girnimbine is found to have higher dock score and its molecular properties, ADME and toxicity profiles were also acceptable so analogs were prepared for it and further computational analysis was carried out on them. A comparison of the relative binding affinities for structurally similar compounds to girnimbine indicates that these computational methods gave suitable analogs. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis which also minimizes killing of animals, wastage of chemicals and also reduces the time and cost for the drug discovery process.

The present study demonstrated that analog V and analog VIII are having better binding interactions with insulin receptor when compared to parent compound girnimbine and other analogs. Other analogs having high binding energy than girnimbine are analogs IV, II, VI, VII, IX, XIV, III and XII. The binding energies of the protein- ligand interactions also confirmed that the ligands will fit into the active pockets of receptor tightly. Even by considering the ADME & T profiles, respective analogs have better profiles when compared to other analogs. These may hold better potential as drug candidates for diabetes. Further these have to be prepared, tested and analyzed in the laboratory for generation of novel high potent antidiabetic drugs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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