Available Online at www.jddt.in

ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 2 (23) 2014, 24-31

RESEARCH ARTICLE

Docking and QSAR based screening of some naturally occurring diterpenes as inhibitors of angiotensin converting enzyme (ACE) against cardiovascular diseases

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Received 15 November 2014; Accepted 25 November 2014

ABSTRACT

Background: Many plants have been listed in the traditional systems of medicine and some of these are providing comprehensive relief to the people suffering from cardio-vascular diseases (CVD) and are recognized for their ability to produce secondary metabolites. Secondary metabolites obtained from different plants have been the starting material for designing different drugs. Naturally occurring diterpenes are the secondary metabolites that show myriad varieties of pharmacological activities. These diterpenes exert cardiovascular actions and paved the path towards development of cardioprotective formulations.

Aims and objectives: In the present study we have analyzed the inhibitory potential of naturally occurring diterpenes on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases.

Materials and methods: Binding affinity of the diterpenes against known cardioprotective drug target was calculated by performing the docking experiment using FlexX and IC50 values of the compounds were predicted by QSAR analysis.

Results: Toxicity screening revealed that diterpenes were non-toxic and obeyed Lipinski's rule. The docking studies showed greater affinity of the diterpenes towards the active site of drug target. QSAR analysis revealed significant IC 50 values of the diterpenes.

Conclusion: The study suggests that the diterpenes - Ent-kaur-16-en-15-one-19-oic acid, may be Angiotensin Converting Enzyme targeted potent new drug for treating cardiovascular diseases.

Keywords: Secondary metabolites, diterpenes, ACE, Cardioprotective, IC50

INTRODUCTION:

Cardiovascular disease (CVD) refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease. Diseases of the cardiovascular system include those that compromise the pumping ability of the heart, cause failure of the valves, or result in narrowing or hardening of the arteries. Injury or failure of the cardiovascular system, especially the heart, also affects the peripheral tissues that depend on the delivery of nutrients and the removal of wastes through the blood vascular system. CVD is a family of diseases that includes hypertension, atherosclerosis, coronary heart disease, and stroke [1]. Angiotensin Converting Enzyme is the prime target for preventing CVD as the enzyme catalyses conversion of Angiotensin I into Angiotensin II [2]. Angiotensin II is a vasoconstrictor that causes blood vessels to constrict thereby causing hypertension [3]. ACE is expressed in small pulmonary arteries normally. However, during diabetes, obesity, hypertension the expression and activities of the enzyme increases in small pulmonary arteries [4]. This led to the development of ACE inhibitors which show significant cardioprotection through decreasing hypertension [5]. This also relieves other hypertension linked ailments like kidney diseases, diabetes etc. [6, 23].

Meanwhile, herbal based secondary metabolites are constantly being screened for drug discovery with respect to ACE inhibition. Diterpenes form a large class of plants-derived secondary metabolites that possess a wide spectrum of important biological activities. Many reports have extensively shown that several classes of diterpenes exert significant cardiovascular effects and some of them are Forskolin; 14-deoxy-11, 12-didehydroandrographolide; ent-kaur-16-en-19-oic acid; ent-kaur-16-en-15-one-19-oic acid; 8-(17), 12E, 14-labdatrien-18-oic acid and Labd-8(17)-en-15-oic acid [7, 8]. We, therefore, thought it prudent that the diterpenes may inhibit ACE and thus

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provide cardioprotection. In the present study we have analyzed the inhibitory potential of the diterpenes on the Angiotensin Converting Enzyme (ACE) - the enzyme responsible for various cardiovascular diseases and compare the activities with known angiotensin Converting Enzyme inhibitors.

MATERIALS AND METHODS:

The Ligands:

Six naturally occurring diterpenes were selected for study using available literature [9], the structure of the ligands were drawn using Chemsketch [10], a chemically intelligent drawing interface freeware was used to construct the structure of the ligands. The three dimensional structures of the compounds in PDB formats were generated and converted to SMILES using OpenBabel [11] and then converted to .sdf format again using OpenBabel. Known ACE inhibitors Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril were used as reference [22]. The structures of these inhibitors were obtained from NCBI PubChem Compound (http://www.ncbi.nlm.nih.gov/pccompound).

ADME/Tox Screening:

ADMET screening helps in detecting drug likeliness of compounds [12]. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) screening was done using Mobyle@rpbs server [13]. The compounds were loaded in the server in SMILES format based on the following parameters:

Molecular weight : min 200.0 max 500.0, Hydrogen donors : min 0.0 max 5.0, Hydrogen acceptors : min 0.0 max 10.0, Flexible bonds : min 0.0 max 15.0, Rigid bonds : min 0.0 max 50.0, Ring number : min 0.0 max 7.0, Ring size : min 0.0 max 12.0, No. of Carbons: >2, Hetero atoms: >2, Ratio carbon/hetero : min 0.1 max 1.0, Charge number : min 0.0 max 3.0, Total charge : min -2.0 max 2.0, logP : min -2.0 max 6.0, Polar Surface Area : min 0.0 max 150.0

The receptor:

The crystal structure of the drug target Angiotensin Converting Enzyme (PDB ID: 1O8A) was obtained from RCSB Protein Data Bank (http://www.rcsb.org). The protein has one chain (Chain A) of 589 residues determined by X-ray diffraction method at a resolution of 2.00 Å. It was deposited by: Natesh et al., in the year 2002.

Active site identification:

The PDB file was loaded into Q-Site Finder to identify the active site amino acids at default parameter setting [14].

Protein – Ligand interaction using FlexX:

Docking is a term used for computational schemes that attempt to find the best matching between two molecules: a receptor and ligand. The receptor Angiotensin Converting Enzyme (ACE) was docked with the known ACE inhibitors and the diterpenes using software FlexX [15, 16]. The active site amino acids were defined in the target molecule during the target preparation and residues within a rdius of 10 Å were included within binding site. The SDF file of all the compounds was loaded in FlexX as docking library. The output file gave the energy values in Kcal/mol. For each docked molecule, this value was noted down.

Quantitative Structure Activity Relationship (QSAR) studies:

The QSAR analysis was performed by taking the known Angiotensin Converting Enzyme inhibitors *viz.* Benazepril, Captopril, Enalapril, Fosinopril, Imidapril, Lisinopril, Quinapril, Ramipril, Trandolapril and Zofenopril.

The QSAR descriptors *viz.* Polarizability, Molar Refractivity, Molar volume, Molecular weight and LogP were generated for each of the molecule using ACD ChemSketch softwares [17]. The activities have been calculated by taking the inverse logarithm of IC50 values. The descriptors were tabulated in a MS Excel Sheet against their bioactivities (log IC_{50}^{-1}). The descriptors and activities were loaded in Easy QSAR software for multiple linear regression analysis. From the regression, the QSAR equation was generated and the activities of t were predicted [18].

RESULTS:

Angiotensin Converting Enzyme, which is a target for cardiovascular disease was selected based on the literature survey. The structure of Angiotensin Converting Enzyme was obtained in PDB format. The active site characterization of the enzyme using Q-site finder showed that HIS383, GLU403, HIS387, GLU411, HIS353, ARG522, VAL518, MET223, GLN281, GLY404, ALA356, LYS511, GLU162, ASP377 and ASP415 are the key amino acids forming active site.

For any molecule to become a drug it should not have any toxic or allergenic effects and it should possess all the ADME/Tox properties. The ADME/Tox screening of the compounds has not shown any negative results, which indicates the potentiality of these molecules to become drugs. The results of the ADME/Tox screening are described in table 1.

Interaction energies between ligand and receptor play the most crucial role in drug designing. In this work, Angiotensin Converting Enzyme (PDB ID: 1O8A) was selected as drug target and the interactions of the compounds were studied using FlexX. The docking results

of both diterpenes and known ACE inhibitors are described in table 2 and table 3 respectively. (Figure no 1) The QSAR analysis of all the compounds showed significant correlation with R square value of 94.49% (The Rsq value should be definitely high for a good QSAR equation, Higher Rsq means higher fitting of the equation to the given data, Hence better predictions it will provide for new test data). The Adjusted Rsq is 85.31 % therefore the difference between Rsq and adjusted Rsq is less (High difference in Rsq and Adjusted Rsq indicates weaker overall prediction). The F statistics of the test is 10.29 and the critical F is 3.69 (The F statistics of the test should be greater than Critical F otherwise the generated equation is inefficient) [19].

The equation generated out of QSAR analysis is as follows:

Activity = 2.90 -0.168 Molar Volume) + 0.117 (Parachor) + 0.160 (Molar refractivity) + 1.68 (LogP) -0.179 (Molecular Weight). (Figure no 2)

From the above QSAR equation the IC 50 values of all the diterpenes were predicted and the values are 1.69 nM, 7.41 nM, 1.99 nM, 1.90 nM, 0.48 nM and 3.46 nM for 8-(17), 12E, 14-labdatrien-18-oic acid; 14-deoxy-11, 12-didehydroandrographolide; Ent-kaur-16-en-19-oic acid; Ent-kaur-16-en-15-one-19-oic acid; Forskolin and Labd-8(17)-en-15-oic acid respectively.

Comparative analysis ACE inhibitory potential of diterpenes and known inhibitors: From the docking score of diterpenes and the known inhibitors it was found that diterpenes - Ent-kaur-16-en-15-one-19-oic acid have much more binding affinity compared to the known ACE inhibitors and the IC 50 values of Ent-kaur-16-en-15-one-19-oic acid is 1.90 nM.

Table no 1: ADMET Properties of naturally occurring Diterpenes

Parameters	**MW**	**Drs**	**Ars**	**FB**	**RB**	**#R**	**RL**	**C**	**H**	**C/H**	**#Chrg**	**Chrg**	**LogP**	**PSA**
Parameter standards	**200-500**	**0-5**	**0-10**	**0-15**	**0-50**	**0-7**	**0-12**	**5-12**	**>2**	**0.1-1.0**	**0-3**	**(-2)-2**	**(-2)-6**	**0-150**
8-(17), 12E, 14-labdatrien-18-oic acid	311.3	5	8	9	8	1	6	10	8	0.8	1	1	0.53	128.63
14-deoxy-11, 12-didehydroandrographolide	302.2	5	7	1	18	3	6	11	9	0.81	0	0	2.82	127.45
Ent-kaur-16-en-19-oic acid	318.2	4	8	7	18	3	6	12	9	0.75	0	1	3.06	147.68
Ent-kaur-16-en-15-one-19-oic acid	456.3	2	3	6	27	5	6	9	3	0.33	2	0	6.75	57.53
Forskolin	384.3	0	6	5	23	4	6	12	8	0.66	0	0	2.42	59.08
Labd-8(17)-en-15-oic acid	482.3	4	10	4	28	5	6	12	4	0.33	3	1	1.9	166.14

MW : Molecular weight, Drs : Donors, Ars : Acceptors, FB : flexible bonds, RB : Rigid Bonds, #R : Ring Number, RL : Ring Length, C : carbons, H : Hetero atoms, C/H : ratio Hetero atoms/carbons, #Chrg : number of charges, Chrg: Total Charge, LogP : logP (octanol / water), PSA: Polar surface area.

Table no 2: Docking result of naturally occurring Diterpenes

Compounds	Total Score (Kcal/mol)	Hydrogen Bond Properties	
		Hydrogen Bonds	Bond Energy (Kcal/mol)
8-(17), 12E, 14-labdatrien-18-oic acid	-20.0064	LYS 511 - O3	-7.7
		TYR 520 - O3	-4.7
		GLN 281 - O1	-2.2
		GLN 281 - O3	-2.8
14-deoxy-11, 12-didehydroandrographolide	-20.3828	ALA 354 - H47	-4.7
		GLU 411 - H 41	-4.4
		TYR 523 - O12	-2.8
		GLU 411 - O 12	-3.1
		ASP 358 - O24	-4.7
Ent-kaur-16-en-19-oic acid	-17.9338	ARG 124 - O3	-8.3
		ARG 124 - O1	-7.4
Ent-kaur-16-en-15-one-19-oic acid	-28.6609	HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-2.9
		HIS 387 - O4	-4.7
		GLU 411 - H59	-4.3
		ARG 522 - O3	-3.0
		ALA 356 - H52	-4.1
Forskolin	-11.4792	ALA 356 - H63	-3.8
		ALA 356 - O3	-3.9
		ASN 66 - O19	-4.7
Labd-8(17)-en-15-oic acid	-16.1402	ARG 124 - O1	-8.3
		ARG 124 - O1	-4.5
		ARG 124 - O3	-8.3

Table no 3: Docking result of known Angiotensin Converting Enzyme inhibitors

Compounds	Total Score (Kcal/mol)	Hydrogen Bond Properties	
		Hydrogen Bonds	Bond Energy (Kcal/mol)
Benazepril	-13.1522	GLU 411 - H 38	-4.5
		GLU 411 - O 1	-4.3
		HIS 383 - O 1	-4.7
		HIS 513 - O 4	-4.6
		HIS 387 - H 59	-2.2
Captopril	-21.8193	HIS 383 -O 2	-4.7
		GLU 411 - O2	-4.7
		TYR 523 - O2	-3.5
		HIS 513 - O3	-2.6
		HIS 353 - O3	-4.7
Enalapril	-23.6967	GLU 384 - O4	-3.6
		HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-4.0
		GLU 411 - H55	-3.2
		ARG 522 - O3	-4.0
Imidapril	-13.0579	HIS 383 -O 5	-2.5
		GLU 411 - O5	-4.7
		GLU 384 - O3	-4.7
		TYR 523 - O5	-2.4
Lisinopril	-22.7837	HIS 383 -O 5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-2.9
		HIS 387 - O4	-4.7
		GLU 411 - H59	-4.3
		ARG 522 - O3	-3.0
		ALA 356 - H52	-4.1
Perindopril	-15.3663	HIS 383 - O2	-4.7
		GLU 411 - O2	-4.7
		GLU 384 - O3	-4.7
		HIS 513 - O5	-4.5
		ALA 354 - H58	-4.4
Quinapril	-17.6071	HIS 383 - O3	-4.7
		GLU 411 - O3	-3.8
		TYR 523 - O3	-2.8
		GLU 384 - O2	-4.2
		ALA 356 - O5	-2.5
Ramipril	-14.2360	HIS 383 - O5	-4.7
		GLU 411 - O5	-4.7
		TYR 523 - O5	-3.8
		GLU 384 - O4	-3.5
		HIS 357 - H43	-2.7
Trandolapril	-2.9183	TYR 523 - O3	-4.6
		GLU 384 - O2	-4.7
Zofenopril	-14.6572	HIS 383 - S1	-2.9
		GLU 411 - S1	-2.7
		TYR 523 - S1	-3.1
		ALA 356 - O4	-4.0
		ARG 522 - O6	-3.2

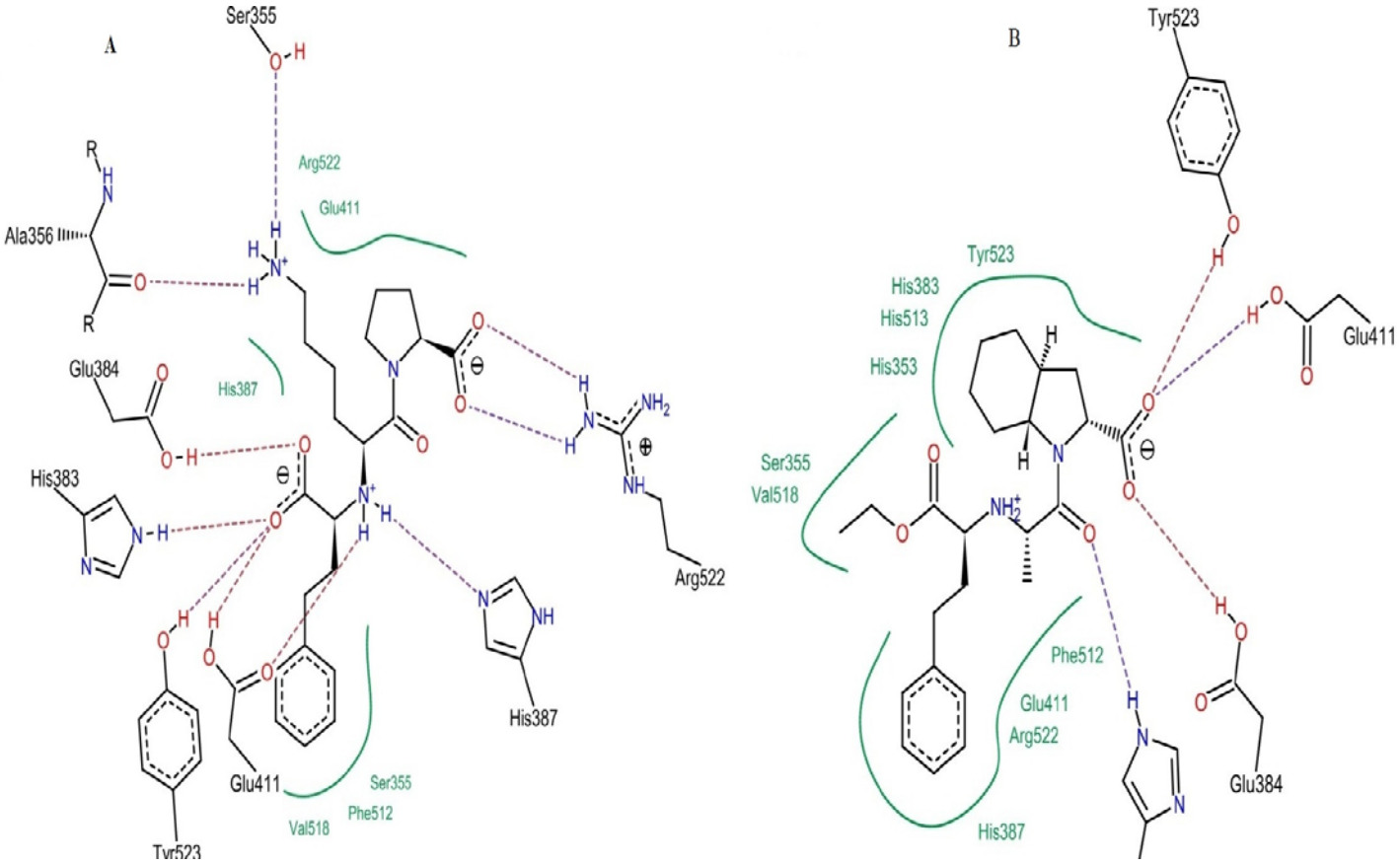

Figure 1:

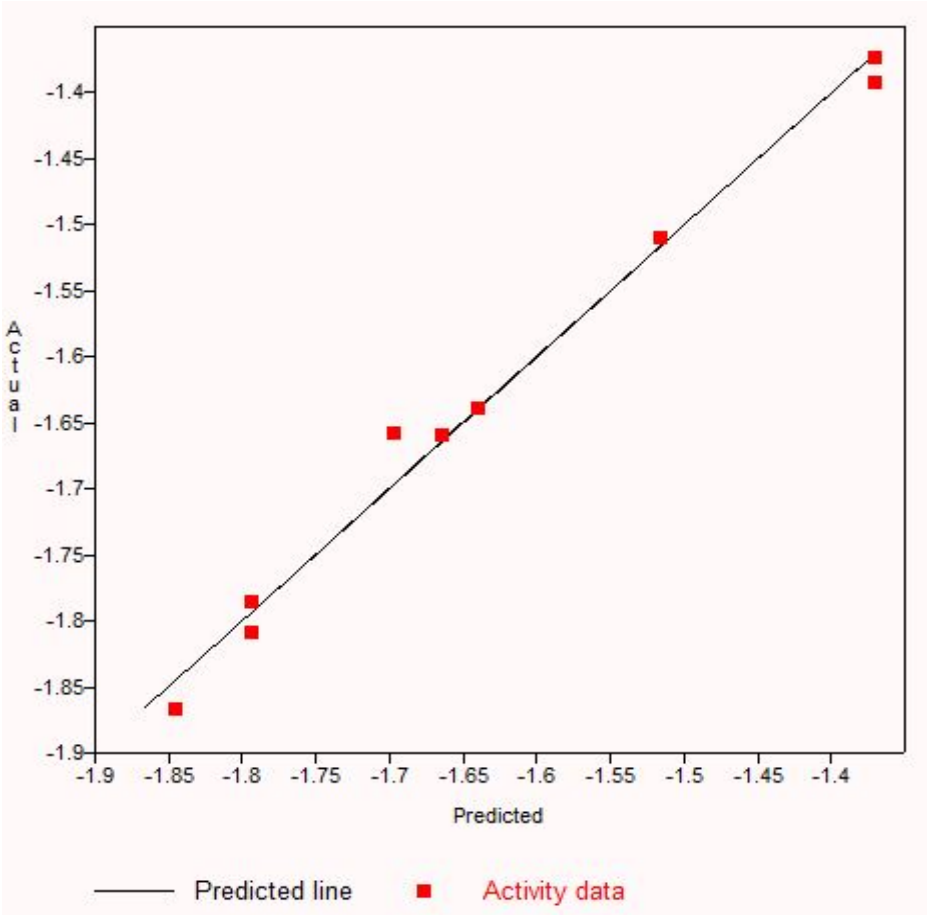

Figure 2:

DISCUSSION:

The ADME/Tox screening of the diterpenes has not shown any negative results, which indicates the potentiality of these molecules to become drugs.

While considering better ligands, the least score in docking was preferred as it indicates more stability in binding [15]. The interaction of the diterpenes were screened based on hydrogen bonding based prediction [20] which shows they binds to the active site residues i.e., HIS383, GLU403, HIS387, GLU411, HIS353,ARG522, VAL518, MET223, GLN281, GLY404, ALA356, LYS511, GLU162, ASP377, ASP415 etc. which was confirmed by the bonded residues in Flex-X.

The interactions of the diterpene - Ent-kaur-16-en-15-one-19-oic acid have much more binding affinity compared to all the known ACE inhibitors

Activity of compound in question has been predicted from QSAR model [21] as inverse logarithm of IC50. It showed that the IC50 of the diterpenes were better than the known inhibitors.

After choosing diterpene - Ent-kaur-16-en-15-one-19-oic acid as better option on the basis of docking score, IC50 and bonding pattern, cross validation was done by target fishing using Pharm mapper software and found that the target comes in suitable range. This analysis indicates suitability of the chosen ligand for the target in one hand and validates the docking result obtained from Flex X.

Angiotensin Converting Enzyme (ACE) produces Angiotensin II - a very potent chemical that causes hypertension. By decreasing the production of angiotensin II through inhibiting the activity of the enzyme ACE, the function of a failing heart can be improve and thus the chances of hypertension and other CVDs can be reduce. Since, diterpene - Ent-kaur-16-en-15-one-19-oic acid binds to the active sites of the enzyme ACE and forms stable bonds therefore, diterpene - Ent-kaur-16-en-15-one-19-oic acid may be used as Angiotensin Converting Enzyme inhibitor. Diterpene - Ent-kaur-16-en-15-one-19-oic acid shows stable bonding pattern in compare to known inhibitors as they shows least score in docking, forms maximum number of hydrogen bonds with the active residues of the enzyme, therefore diterpene - Ent-kaur-16-en-15-one-19-oic acid have more ACE inhibitory potentials.

CONCLUSION:

Based on present observation of docking score of both diterpenes and known inhibitors, IC50 value of known inhibitors and predicted IC50 of the diterpenes we suggests that diterpene - Ent-kaur-16-en-15-one-19-oic acid may be Angiotensin Converting Enzyme targeted potent new drug for treating Cardiovascular diseases. However, further studies are required to validate the same *in vivo* or *in vitro*.

ACKNOWLEDGEMENTS:

The authors are thankful to Department of Biotechnology (DBT), Govt. of India, New Delhi for establishing Bioinformatics Centre in Assam University, Silchar where the work has been carried out. The e-journal access facility provided by Bioinformatics centre, Assam University funded by Department of Biotechnology, Govt. of India is highly acknowledged.

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