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IN SILICO INHIBITION OF PROTO-ONCOGENE TYROSINE PROTEIN KINASE SRC (C-SRC) BY ARISTOLACTAM A II, 4-ALLYL RESORCINOL AND STIGMAST-4-EN-3, 6-DIONE FOR ANTICANCER ACTIVITY OF *PIPER BETLE* LINN. (PIPERACEAE): TRADITIONAL CLAIM REVALIDATED

Paulomi Paul, Abhishek Chowdhury* and Manabendra Dutta Choudhury

Bioinformatics Centre (DBT-BIF), Assam University Silchar, Assam-788011, India.

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***Correspondence for Author**
Abhishek Chowdhury
Bioinformatics Centre (DBT-BIF), Assam University Silchar, Assam-788011 India.

ABSTRACT

Plant based compounds serve as curing agents of various disease for decades. Virtual screening using computational tools provide a wide platform of plant based bioactivity prediction. The current study provides molecular mechanism of compound isolated from roots of *Piper betle*. This work comprises of target prediction and molecular mechanism of Aristolactam A II (ARL), 4-allyl resorcinol (ARL) and Stigmast-4-en-3, 6-dione (STM), compounds isolated from *P. betle* root. The target validation showed proto-oncogene tyrosine protein kinase Src (c-Src) and cyclin dependent kinases (CDK) as the top-ranked targets. Molecular docking of ARL showed good score against c-Src (-17.88 Kcal/mol) and CDK2 (-16.96 Kcal/mol) using Biosolveit LeadIT. Autodock 4.2 validation also showed similar amino acid pattern. Comparison with known c-Src inhibitor Bosutinib (BST) (-27.70 Kcal/mol) and CDK2 inhibitor Staurosporine (STP) (-35.20 Kcal/mol) showed similarity in binding pattern. Toxicity study showed ARL was non-toxic whereas Bosutinib (BST) was reported with high side-effect and toxicity. This concludes that these compounds works as some of the factors to support the ethnomedicinal claim of anticancer activity of *P. betle*.

KEYWORDS: *Piper betle*; Aristolactam A II; 4-allyl resorcinol; Stigmast-4-en-3,6-dione; proto-oncogene tyrosine protein kinase Src; Cyclin dependent kinase 2; c-Src inhibitor; CDK2 inhibitor; molecular docking.

INTRODUCTION

Plant based medicines provided wide range of information pertaining to cure of various ailments.[1] Plants have wide variety of chemical compounds that are used to perform important biological functions.[2] As per the records of the World Health Organization (WHO), 80% of Asian and African population presently relies on herbal medicine for primary health care.

Till date, plant species provides new chemical compounds with structural novelty that helps diverse treatment regimens.[2] Apart from this resistance to drugs, nosocomial infections have called for search of novel compounds.[3-4] Computational tools have added boost to the design and development of novel drug molecules.[5]

It is notable that proper drug target selection is a must for effective drug design.[6] Induced by this state of affairs, SwissTargetPrediction server has been employed as primary tool to find out the possible target by high-throughput screening.[7] This protein target relevant screening of ligand completely elicit the mechanism of the therapeutic properties of molecule.

In silico molecular docking which is a must for drug discovery process brings into lights the presumed binding modes and affinities of the compound with its target. This molecular recognition is important for understanding the mechanism of protein-ligand interaction and the design of therapeutic processes.[8]

Indian ancient culture witnessed the vital role played by *Piper betle*. Indian traditional books of Ayurveda, Charaka Samhitas, Sushruta Samhitas, and Kashyapa Bhojanakalpa enlisted the practice of chewing *P. betle* leaf (BL) after meals.

In Chinese folk medicine, BL are used to treat various diseases and claimed to possess antioxidation and antimutation properties. Patents were also awarded for its anti-inflammatory, anti-cancer, and immunomodulatory activities.[9]

BL was suggested to be harmless when consumed alone as per many researches. Bhide *et al.*[10] suggested that, when aqueous BL extract where administered alone with areca nut extract in mice, they developed different types of cancer while mice fed with only aqueous BL extract did not develop any tumors. Shetty *et al*.[11] have showed the advantage of BL in maintaining salivary ascorbic acid levels in oral cavity of humans. Salivary ascorbic acid may

help prevent carcinogenesis in the oral cavity.[11] However betel roots were of less importance to study in terms of therapeutic benefits.

Hence, the presence study aims to depict the potential role of *P. betle* roots phytochemicals against different diseases. The study deals with *in silico* activity prediction and molecular docking of Arostolactam A II, 4-Allyl Resorcinol and Stigmast-4-En-3, 6-Dione isolated from *P. betle* root.[12]

MATERIALS AND METHODS

Ligand Identification

Three compounds were reported from *Piper betle* root in the work of Ghosh and Bhattacharya, 2005.[12] The compounds were retrieved from PubChem Compound database by searching their name and the SMILES strings were saved. The compounds selected were Aristololactam A II, 4-allyl resorcinol and Stigmast-4-en-3, 6-dione.

Virtual Target Scanning

Reverse docking approach was used using SwissTargetPrediction server[7] by submitting the SMILES of the compounds. The results were saved in terms of hit identified with various targets of *Homo sapiens* genome.

Ligand Selection

Final ligands for the study were selected based on the target scanning report that contained target for cancer prevention. Known inhibitor for the selected targets was also retrieved from PubChem based on various literature searches.

Target Selection

Based on primary target scan report and literature survey proto-oncogene tyrosine protein kinase Src (c-Src) and Cyclin dependent kinase 2 (CDK2) was selected based on their report of anticancer activity and listing in target prediction report of the selected ligands. The structures of the targets were retrieved from PDB (c-Src with PDB ID: 4MXX and CDK2 with PDB ID: 1AQ1).

Molecular docking using Biosolveit LeadIT

i. Preparation of protein

Protein 3D structure for the target was retrieved from Protein DataBank (PDB). The structure was then submitted to LeadIT protein loading platform. Chain A was selected for the protein with no co-factor and small bound molecules.

ii. Active site determination and processing

Active site was determined based on the architecture shown by bound ligand with radius of 6.5Å. The water molecules were removed to prevent unwanted iteration with surrounding water. Specific residues were marked based on literature survey. Finally the protein model was fixed for further analysis.

iii. Ligand preparation

Ligands were merged into single combined file using freeware OpenBabel in .sdf format. The ligands were protonated; polar hydrogens were added and ionized as in aqueous medium.

iv. Docking parameters

The docking parameters like hits per search, hit by iteration were kept at 20-200 range and all others values were set as default.

v. Pharmacophore generation

Finally, the poses to be generated were set as 10 and docking was performed. The results were noted down and docking poses were saved as image file in .png format.

Energy minimization and stabilization

Energy minimization was performed for the protein target using gromacs steepest descent method and ligand using PRODGR server. The above five steps were repeated and the results were noted as above.

Comparison with known inhibitor

The same was followed for selected known inhibitors and were compared to find pattern similarity and binding affinity.

Result

The selected ligands (Figure1) obtained from roots of *Piper betle* after spectroscopic study of alcoholic and petroleum-ether extracts.[12]

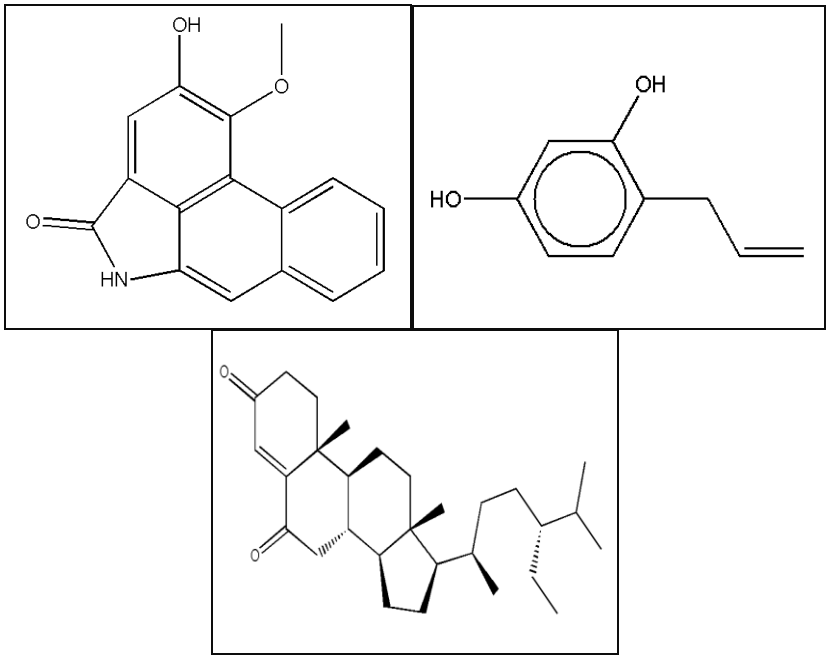

Figure1. Structure of Aristolactam A II, Stigmast-4-en-3,6-dione isolated from *Piper betle* and c-Src inhibitor Bosutinib (from left to right).

The structure shows aromatic compounds with hydrogen donors and acceptors which are required for druglikeness.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cyclin-dependent kinase 1	P06493	CDK1	CHEMBL308		30 / 6	Ser_Thr Kinase
Cyclin-dependent kinase 4	P11802	CDK4	CHEMBL331		30 / 6	Ser_Thr Kinase
Cyclin-dependent kinase 2	P24941	CDK2	CHEMBL301		30 / 6	Ser_Thr Kinase
Cyclin-dependent kinase 3 *(by homology)*	Q00526	CDK3	CHEMBL4442		30 / 6	Ser_Thr Kinase
Cyclin-dependent kinase 6 *(by homology)*	Q00534	CDK6	CHEMBL2508		30 / 6	Ser_Thr Kinase
Aurora kinase A	O14965	AURKA	CHEMBL4722		14 / 4	Ser_Thr Kinase
Aurora kinase B	Q96GD4	AURKB	CHEMBL2185		14 / 4	Ser_Thr Kinase
Aurora kinase C *(by homology)*	Q9UQB9	AURKC	CHEMBL3935		14 / 4	Ser_Thr Kinase
FAD-linked sulfhydryl oxidase ALR	P55789	GFER	CHEMBL1741189		48 / 1	Enzyme
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		198 / 12	Unclassified
Tyrosine-protein kinase Fyn *(by homology)*	P06241	FYN	CHEMBL1841		26 / 10	Tyr Kinase
Tyrosine-protein kinase Yes *(by homology)*	P07947	YES1	CHEMBL2073		26 / 10	Tyr Kinase
Tyrosine-protein kinase Fgr *(by homology)*	P09769	FGR	CHEMBL4454		26 / 10	Tyr Kinase
Proto-oncogene tyrosine-protein kinase Src	P12931	SRC	CHEMBL267		26 / 10	Tyr Kinase
Tyrosine-protein kinase FRK *(by homology)*	P42685	FRK	CHEMBL4223		26 / 10	Tyr Kinase

Figure2. SwissTargetPrediction result of Aristolactam A II.

The results highlight the presence of Cyclin-dependent kinase 2 (CDK2) and Proto-oncogene tyrosine-protein kinase Src (cSrc), two important targets of various types of cancer.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Serine/threonine-protein kinase 25	O00506	STK25	CHEMBL5552		6 / 3	Ser_Thr Kinase
Citron Rho-interacting kinase	O14578	CIT	CHEMBL5579		5 / 3	Ser_Thr Kinase
Serine/threonine-protein kinase RIO3	O14730	RIOK3	CHEMBL5659		4 / 1	Ser_Thr Kinase
Dual specificity mitogen-activated protein kinase kinase 7	O14733	MAP2K7	CHEMBL3530		3 / 2	Ser_Thr_Tyr Kinase
Serine/threonine-protein kinase Chk1	O14757	CHEK1	CHEMBL4630		271 / 4	Ser_Thr Kinase
Peripheral plasma membrane protein CASK	O14936	CASK	CHEMBL1908381		1 / 1	Enzyme
Cyclin-G-associated kinase	O14976	GAK	CHEMBL4355		9 / 4	Ser_Thr Kinase
Ephrin type-B receptor 6	O15197	EPHB6	CHEMBL5836		29 / 5	Unclassified
Mitogen-activated protein kinase kinase kinase 13	O43283	MAP3K13	CHEMBL1163124		4 / 2	Ser_Thr Kinase
Mitogen-activated protein kinase kinase kinase 7	O43318	MAP3K7	CHEMBL5776		5 / 1	Ser_Thr Kinase
Receptor-interacting serine/threonine-protein kinase 2	O43353	RIPK2	CHEMBL5014		2 / 2	Ser_Thr Kinase
NUAK family SNF1-like kinase 1	O60285	NUAK1	CHEMBL5784		8 / 4	Ser_Thr Kinase
Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma *(by homology)*	O60331	PIP5K1C	CHEMBL1908383		5 / 1	Enzyme
Tyrosine-protein kinase JAK2	O60674	JAK2	CHEMBL2971		79 / 3	Tyr Kinase
Rho-associated protein kinase 2	O75116	ROCK2	CHEMBL2973		111 / 2	Ser_Thr Kinase

Figure3. SwissTargetPrediction result of c-Src inhibitor Bosutinib.

The result highlights the presence of various protein kinases like serine threonine kinases and tyrosine kinases.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		288 / 3	Unclassified
cAMP-dependent protein kinase catalytic subunit alpha	P17612	PRKACA	CHEMBL4101		4 / 7	Ser_Thr Kinase
cAMP-dependent protein kinase catalytic subunit beta	P22694	PRKACB	CHEMBL2918		4 / 7	Ser_Thr Kinase
cAMP-dependent protein kinase catalytic subunit PRKX	P51817	PRKX	CHEMBL5818		4 / 7	Ser_Thr Kinase
cGMP-dependent protein kinase 2	Q13237	PRKG2	CHEMBL2896		4 / 4	Ser_Thr Kinase
cGMP-dependent protein kinase 1	Q13976	PRKG1	CHEMBL4273		7 / 19	Ser_Thr Kinase
cAMP-dependent protein kinase catalytic subunit gamma *(by homology)*	P22612	PRKACG	CHEMBL2743		4 / 7	Ser_Thr Kinase
Serine/threonine-protein kinase Chk1	O14757	CHEK1	CHEMBL4630		57 / 7	Ser_Thr Kinase
Serine/threonine-protein kinase Sgk1 *(by homology)*	O00141	SGK1	CHEMBL2343		4 / 3	Ser_Thr Kinase
Bone morphogenetic protein receptor type-1B	O00238	BMPR1B	CHEMBL5476		14 / 2	Ser_Thr Kinase
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta isoform *(by homology)*	O00329	PIK3CD	CHEMBL3130		133 / 3	Enzyme
Serine/threonine-protein kinase PLK4	O00444	PLK4	CHEMBL3788		4 / 4	Ser_Thr Kinase
Serine/threonine-protein kinase 25	O00506	STK25	CHEMBL5552		4 / 4	Ser_Thr Kinase
Citron Rho-interacting kinase	O14578	CIT	CHEMBL5579		3 / 2	Ser_Thr Kinase
Serine/threonine-protein kinase RIO3	O14730	RIOK3	CHEMBL5659		4 / 4	Ser_Thr Kinase

Figure4. SwissTargetPrediction result of CDK2 inhibitor Staurosporine.

The results highlight the presence of various protein kinases like serine threonine kinases and tyrosine kinases.

Target Selection

The target c-Src has 286 residues and consists of identical chains A and B with 14 helices and 12 strands, while CDK2 has 298 residues and consists of 12 helices and 9 strands.

Ligand Selection

The SwissTargetPrediction server result showed that only Aristololactam A II (ARL) have affinity towards two vital targets for cancer prevention and hence ARL was selected for molecular docking against c-Src and CDK2 and compared with their respective inhibitors.

Table1. ADMET properties of ARL, BST and STP calculated by Mobyle@rpbs portal. The result shows high druglikeness of ARL and toxicity shown by BST and STP.

ID	MW	logP	tPSA	FB	RB	HBD	HBA	Rings	TC	C	nC	Sol
ARL	265.2634	2.95	62.32	1	20	2	4	1	0	16	4	6832.254
BST	530.4462	5.38	84.08	9	24	1	8	3	1	26	10	1182.225
STP	466.531	3.24	74.03	2	37	2	7	1	1	28	7	3528.981

MW: Molecular weight; tPSA: topological polar surface area; FB: Flexible bond; RB: Rigid bond; HBD: Hydrogen bond donor; HBA: hydrogen bond acceptor; TC: total charge; C: carbon; nC: non-carbon, Sol: Solubility (mg/L).

Figure5. ADMET profiling of ARL showing druglikeness.

Only Pfizer 3/75 rule showing problem due to presence of rings.

Figure7. Similar ADMET profiling of BST, STP showing toxicity as per Pfizer 3/75 rule.

Molecular docking results were given below

Compound	LeadIT		Autodock 4.2	
	Score	Bonding Pattern	Score	H-bonds
c-Src (4MXX)				
Aristololactam A II	-17.76	Thr403, Met341, Glu339	-8.26	Met341
Busotinib (BST)	-27.70	Asp348, Met341	-8.80	Met341
CDK2 (1AQ1)				
Aristololactam A II	-21.86	Glu81, Leu83	-7.30	Leu83
Staurosporine (STP)	-35.20	Glu81, Leu83	-8.00	Leu83

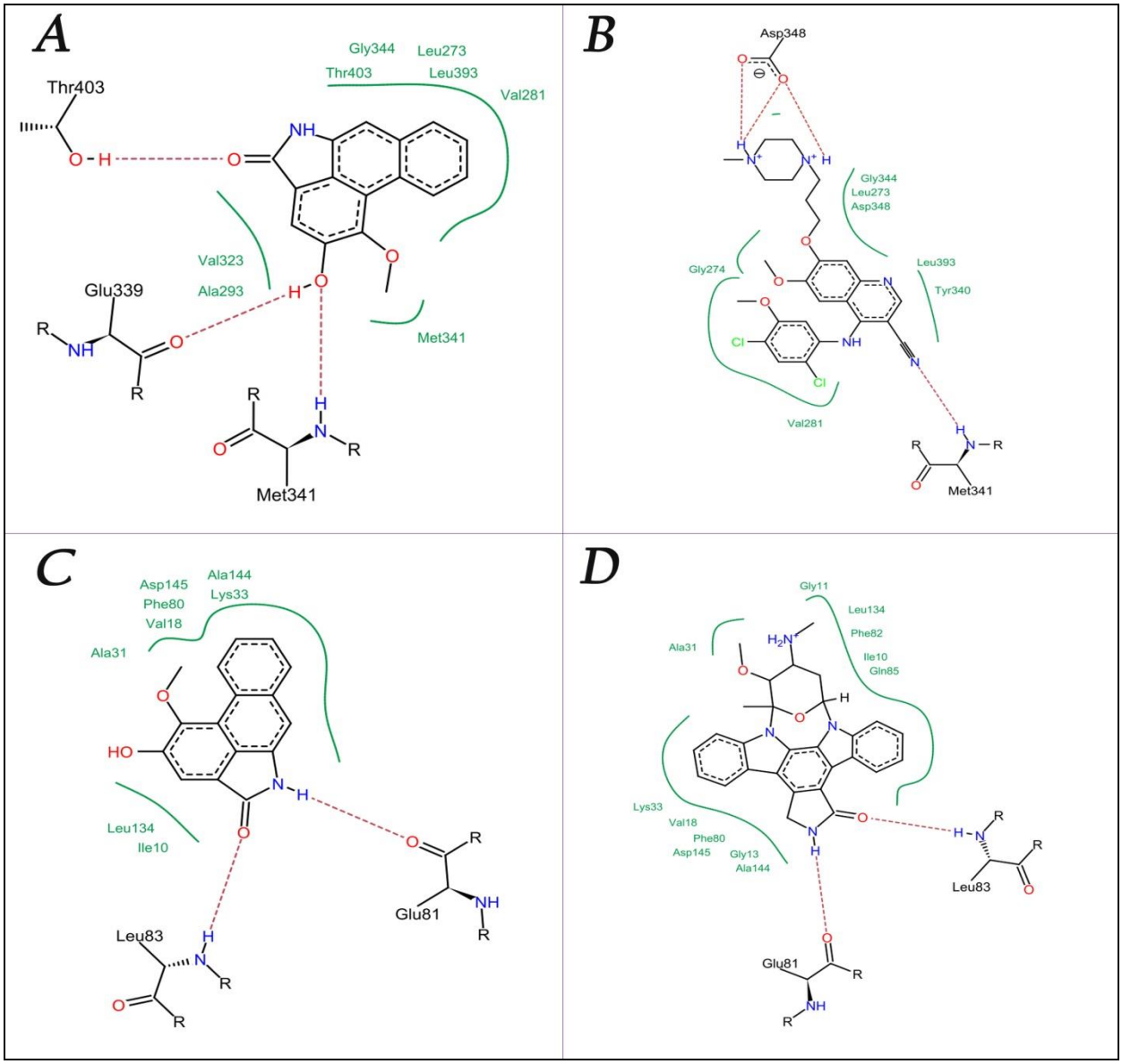

Figure8. Docking pattern of ARL against c-Src (A) showing similar binding pattern to that of BST (B) and ARL against CDK2 (C) showing similarity with STP (D) in LeadIT.

DISCUSSION AND CONCLUSION

Proto-oncogene c-Src or c-Src is a non-receptor tyrosine kinase protein encoded by the SRC gene in human, phosphorylates specific tyrosine in other proteins. It has been seen that increase in the activity of c-Src tyrosine kinase leads to progression of cancer by promoting various other signals. Whereas, CDK-2 or cyclin dependent kinase-2 is an enzyme that is restricted to the G1-S phase of cell cycle and essential for the transition from G1 to S phase. Presence of these targets in ARL target scan supported the presence of anticancer property in this compound.

Molecular docking showed that ARL has docking score very close to that of inhibitors with identical docking pattern. Autodock 4.2 showed the presence of similar trends.

Molecular dynamics simulation was performed upto minimization step and found that the pharmacophore generated was highly stable.

ADMET screening showed that ARL was non-toxic with high solubility whereas, the known inhibitors were highly toxic and with adverse effects. This finding was also supported by the bioactivity of inhibitors in PubChem Bioassay and literature report of ARL.[10-11]

The above findings depict that ARL has anticancer properties and is responsible for the validation of ethnomedicinal claim of *Piper betle* as anticancer medicinal plant.

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